

\*\*\*\*\* STN Columbus \*\*\*\*\*

FILE 'HOME' ENTERED AT 10:29:31 ON 02 OCT 2002

=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull

=> e king kendall w/au

E1 2 KING KEN RICHARD/AU  
E2 1 KING KENDALL A/AU  
E3 41 --> KING KENDALL W/AU  
E4 4 KING KENDALL WAYNE/AU  
E5 15 KING KENDALL WILLARD/AU  
E6 10 KING KENNETH/AU  
E7 2 KING KENNETH A/AU  
E8 2 KING KENNETH BRYAN/AU  
E9 7 KING KENNETH F/AU  
E10 1 KING KENNETH FLOYD/AU  
E11 7 KING KENNETH FRANCIS/AU  
E12 3 KING KENNETH G/AU

=> s e3-e4

L1 45 ("KING KENDALL W"/AU OR "KING KENDALL WAYNE"/AU)

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 41 DUP REM L1 (4 DUPLICATES REMOVED)

=> s l2 and (mycoplasm? or mhp?)

5 FILES SEARCHED...

L3 12 L2 AND (MYCOPLASM? OR MHP?)

=> d bib ab kwic 1-

YOU HAVE REQUESTED DATA FROM 12 ANSWERS - CONTINUE? Y/(N):y

L3 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1997:118765 BIOSIS

DN PREV199799425268

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's  
vaccine potential.

AU \*\*\*King, Kendall W. (1)\*\*\* ; Faulds, Daryl H.; Rosey, Everett L.;  
Yancey, Robert J., Jr.

CS (1) 213 Schuring Road, Portage, MI 49024 USA

SO Vaccine, (1997) Vol. 15, No. 1, pp. 25-35.

ISSN: 0264-410X.

DT Article

LA English

AB The gene encoding \*\*\*Mhp1\*\*\*, a 124 kDa protein from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
product characterized. No significant homology to the gene or encoded  
polypeptide was found in the Genbank, NBRF, or PIR databases, though this  
protein appears similar to p97, a putative adhesin of M. hyopneumoniae  
described by Zhang et al. (Infect. Immun. 63, 1013-1019, 1995). Two  
repeated motifs were identified within the 3' end of the gene and encoded  
polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
expression and purification of the protein. Both the authentic and  
recombinant proteins were recognized by sera from pigs infected with M.  
hyopneumoniae. In an induced-disease model in pigs, coughing was reduced  
in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\*, although  
differences were not significant. Only minimal protection against lung  
lesion formation was provided, and again differences between the

\*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's  
 vaccine potential.

AU \*\*\*King, Kendall W. (1)\*\*\* ; Faulds, Daryl H.; Rosey, Everett L.;  
 Yancey, Robert J., Jr.

AB The gene encoding \*\*\*Mhp1\*\*\* , a 124 kDa protein from  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
 product characterized. No significant homology to the gene or encoded  
 polypeptide was found. . . Immun. 63, 1013-1019, 1995). Two repeated  
 motifs were identified within the 3' end of the gene and encoded  
 polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
 S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
 expression and purification of the protein.. . . from pigs infected  
 with M. hyopneumoniae. In an induced-disease model in pigs, coughing was  
 reduced in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\* ,  
 although differences were not significant. Only minimal protection against  
 lung lesion formation was provided, and again differences between the  
 \*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

BC \*\*\*Mycoplasmataceae\*\*\* 07512  
 Suidae \*85740

IT Sequence Data  
 amino acid sequence; nucleotide sequence

IT Miscellaneous Descriptors  
 BACTERIAL DISEASE; CHARACTERIZATION; \*\*\*MHP1\*\*\* GENE; \*\*\*MHP1\*\*\*  
 PROTEIN; MOLECULAR GENETICS; \*\*\*MYCOPLASMA\*\*\* HYOPNEUMONIAE  
 INFECTION; PHARMACOLOGY; VACCINE POTENTIAL

ORGN Super Taxa  
 Bacteria - General Unspecified: Eubacteria, Bacteria;  
 \*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria; Suidae: Artiodactyla,  
 Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name  
 bacteria (Bacteria - General Unspecified); pig (Suidae);  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms  
 animals; artiodactyls; bacteria; chordates; eubacteria; mammals;  
 microorganisms; nonhuman mammals; nonhuman vertebrates; vertebrates

L3 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1996:259994 BIOSIS

DN PREV199698816123

TI Analysis of ciliary adhesin protein genes in \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae.

AU Hsu, T. (1); \*\*\*King, Kendall W.\*\*\* ; Minion, F. C.

CS (1) Iowa State Univ., Ames, IA 50011 USA

SO Abstracts of the General Meeting of the American Society for Microbiology,  
 (1996) Vol. 96, No. 0, pp. 283.  
 Meeting Info.: 96th General Meeting of the American Society for  
 Microbiology New Orleans, Louisiana, USA May 19-23, 1996  
 ISSN: 1060-2011.

DT Conference

LA English

TI Analysis of ciliary adhesin protein genes in \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae.

AU Hsu, T. (1); \*\*\*King, Kendall W.\*\*\* ; Minion, F. C.

BC \*\*\*Mycoplasmataceae\*\*\* \*07512  
ORGN Super Taxa  
\*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria  
ORGN Organism Name  
\*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mycoplasmataceae\*\*\* )  
ORGN Organism Superterms  
bacteria; eubacteria; microorganisms

L3 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1996:198072 BIOSIS

DN PREV199698754201

TI Artificial transformation of mollicutes via polyethylene glycol- and electroporation-mediated methods.

AU Dybvig, Kevin (1); Gasparich, Gail E.; \*\*\*King, Kendall W.\*\*\*

CS (1) Dep. Comparative Med., Univ. Ala., Birmingham, AL 35294 USA

SO Razin, S. [Editor]; Tully, J. G. [Editor]. (1995) pp. 179-184. Molecular and diagnostic procedures in mycoplasmaology, Vol. 1. Molecular characterization.

Publisher: Academic Press, Inc. 1250 Sixth Ave., San Diego, California 92101, USA.

ISBN: 0-12-583805-0.

DT Book

LA English

AU Dybvig, Kevin (1); Gasparich, Gail E.; \*\*\*King, Kendall W.\*\*\*

BC \*\*\*Mycoplasmas\*\*\* 07500

Acholeplasmataceae 07511

\*\*\*Mycoplasmataceae\*\*\* 07512

Spiroplasmataceae \*07513

ORGN Super Taxa

Acholeplasmataceae: Eubacteria, Bacteria; \*\*\*Mycoplasmas\*\*\* :

Eubacteria, Bacteria; \*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria;

Spiroplasmataceae: Eubacteria, Bacteria

ORGN Organism Name

Acholeplasma laidlawii (Acholeplasmataceae); \*\*\*Mycoplasma\*\*\*

pulmonis ( \*\*\*Mycoplasmataceae\*\*\* ); \*\*\*Mycoplasmas\*\*\* (

\*\*\*Mycoplasmas\*\*\* ); Spiroplasma citri (Spiroplasmataceae)

ORGN Organism Superterms

bacteria; eubacteria; microorganisms

L3 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1994:299502 BIOSIS

DN PREV199497312502

TI Transformation of \*\*\*Mycoplasma\*\*\* capricolum and examination of DNA restriction modification in M. capricolum and \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides.

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin (1)

CS (1) Dep. Comparative Med., Univ. Ala., Birmingham, AL 35294 USA

SO Plasmid, (1994) Vol. 31, No. 3, pp. 308-311.

ISSN: 0147-619X.

DT Article

LA English

AB Plasmids pIK-DELTA and pIK-DELTA-erm have recently been developed as

\*\*\*mycoplasmal\*\*\* cloning vectors. In this report, we demonstrate that

these plasmids can replicate in \*\*\*Mycoplasma\*\*\* capricolum, a

\*\*\*mycoplasmal\*\*\* species for which transformation had not previously

been characterized. Both plasmids are stably maintained at a higher copy number than in their parental species, \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. We have also examined the possibility of one or more restriction-modification systems affecting transformation frequencies in both species.

TI Transformation of \*\*\*Mycoplasma\*\*\* capricolum and examination of DNA restriction modification in M. capricolum and \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides.

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin (1)

AB Plasmids pIK-DELTA and pIK-DELTA-erm have recently been developed as \*\*\*mycoplasma\*\*\* cloning vectors. In this report, we demonstrate that these plasmids can replicate in \*\*\*Mycoplasma\*\*\* capricolum, a \*\*\*mycoplasma\*\*\* species for which transformation had not previously been characterized. Both plasmids are stably maintained at a higher copy number than in their parental species, \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. We have also examined the possibility of one or more restriction-modification systems affecting transformation frequencies in both. . .

BC \*\*\*Mycoplasmataceae\*\*\* \*07512

ORGN Super Taxa

\*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria

ORGN Organism Name

\*\*\*Mycoplasma\*\*\* capricolum ( \*\*\*Mycoplasmataceae\*\*\* );

\*\*\*Mycoplasma\*\*\* mycoides ssp. mycoides ( \*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms

bacteria; eubacteria; microorganisms

L3 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1994:176580 BIOSIS

DN PREV199497189580

TI Cloning and characterization of the recA genes from \*\*\*Mycoplasma\*\*\* pulmonis and M. mycoides subsp. mycoides.

AU \*\*\*King, Kendall W.\*\*\* ; Woodard, Ann; Dybvig, Kevin (1)

CS (1) Dep. Comparative Med., Univ. Ala. Birmingham, 418A Volker Hall, Birmingham, AL 35294 USA

SO Gene (Amsterdam), (1994) Vol. 139, No. 1, pp. 111-115.

ISSN: 0378-1119.

DT Article

LA English

AB The RecA protein has a central role in DNA repair and is essential for homologous recombination in most eubacteria. Little is known about these critical processes in \*\*\*mycoplasmas\*\*\*. By using standard and inverse polymerase chain reactions (PCR) coupled with conventional cloning techniques, a series of overlapping fragments comprising the entire recA genes of \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides (Mm) and \*\*\*Mycoplasma\*\*\* pulmonis (Mp) were generated. Each gene was sequenced in its entirety. The recA genes of Mm and Mp would encode proteins of 345 amino acids (aa) and 339 aa, respectively. The \*\*\*mycoplasma\*\*\* RecA proteins revealed strong conservation when compared with RecA sequences from other bacterial species.

TI Cloning and characterization of the recA genes from \*\*\*Mycoplasma\*\*\* pulmonis and M. mycoides subsp. mycoides.

AU \*\*\*King, Kendall W.\*\*\* ; Woodard, Ann; Dybvig, Kevin (1)

AB. . . in DNA repair and is essential for homologous recombination in most eubacteria. Little is known about these critical processes in

\*\*\*mycoplasmas\*\*\* . By using standard and inverse polymerase chain reactions (PCR) coupled with conventional cloning techniques, a series of overlapping fragments comprising the entire recA genes of \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides (Mm) and \*\*\*Mycoplasma\*\*\* pulmonis (Mp) were generated. Each gene was sequenced in its entirety. The recA genes of Mm and Mp would encode proteins of 345 amino acids (aa) and 339 aa, respectively. The \*\*\*mycoplasma\*\*\* RecA proteins revealed strong conservation when compared with RecA sequences from other bacterial species.

BC \*\*\*Mycoplasmataceae\*\*\* \*07512

ORGN Super Taxa

\*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria

ORGN Organism Name

\*\*\*Mycoplasma\*\*\* mycoides ( \*\*\*Mycoplasmataceae\*\*\* );

\*\*\*Mycoplasma\*\*\* pulmonis ( \*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms

bacteria; eubacteria; microorganisms

L3 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1994:126462 BIOSIS

DN PREV199497139462

TI \*\*\*Mycoplasma\*\*\* cloning vectors derived from plasmid pKMK1.

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin (1)

CS (1) Dep. Comparative Med., Univ. Ala. Birmingham, Birmingham, AL 35294 USA

SO Plasmid, (1994) Vol. 31, No. 1, pp. 49-59.

ISSN: 0147-619X.

DT Article

LA English

AB Only two plasmids have been isolated and characterized from the entire genus \*\*\*Mycoplasma\*\*\* , which includes over 90 recognized species.

Both of these plasmids were obtained from the same species,

\*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. We have previously characterized one of these plasmids, PKMK1, as a preliminary step in developing \*\*\*mycoplasma\*\*\* cloning vectors. In the present study, we have separately combined pKMK1 with two different Escherichia coli replicons and a tetracycline resistance (tetM) gene. One of the construct, plasmid p2D4, was shuttled from E. coli to M. mycoides subsp. mycoides and back to E. coli with no deletions or rearrangements occurring in the plasmid. In the second construct, the E. coli replicon was deleted when the plasmid was transformed into M. mycoides subsp. mycoides. This derivative, designated plasmid pIK-DELTA, is noteworthy in that it could be transformed into M. mycoides subsp. mycoides at a much higher frequency than the parental plasmid. A gram-positive bacterial erythromycin resistance determinant (erm) was cloned into both p2D4 and pIK-DELTA. Resistance to erythromycin was stably maintained using both constructs, even in the absence of erythromycin selection, indicating that these plasmids will be useful \*\*\*mycoplasma\*\*\* cloning vectors.

TI \*\*\*Mycoplasma\*\*\* cloning vectors derived from plasmid pKMK1.

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin (1)

AB Only two plasmids have been isolated and characterized from the entire genus \*\*\*Mycoplasma\*\*\* , which includes over 90 recognized species.

Both of these plasmids were obtained from the same species,

\*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. We have previously characterized one of these plasmids, PKMK1, as a preliminary step in developing \*\*\*mycoplasma\*\*\* cloning vectors. In the present study, we

have separately combined pKMK1 with two different Escherichia coli replicons and a tetracycline. . . was stably maintained using both constructs, even in the absence of erythromycin selection, indicating that these plasmids will be useful \*\*\*mycoplasmal\*\*\* cloning vectors.

BC Enterobacteriaceae 06702

\*\*\*Mycoplasmataceae\*\*\* \*07512

ORGN Super Taxa

Enterobacteriaceae: Eubacteria, Bacteria; \*\*\*Mycoplasmataceae\*\*\* :  
Eubacteria, Bacteria

ORGN Organism Name

Escherichia coli (Enterobacteriaceae); \*\*\*Mycoplasma\*\*\* mycoides (  
\*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms

bacteria; eubacteria; microorganisms

L3 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2002 ACS

AN 2001:261139 CAPLUS

DN 134:294510

TI Sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and  
therapeutic uses thereof

IN \*\*\*King, Kendall Wayne\*\*\* ; Madura, Rebecca Anne; Rosey, Everett Lee

PA Pfizer Products Inc., USA

SO Eur. Pat. Appl., 38 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI EP 1090995	A2	20010411	EP 2000-308421	20000926
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EP 1090995	A3	20010418		
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO

BR 2000004488	A	20011113	BR 2000-4488	20000927
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CN 1296953	A	20010530	CN 2000-129083	20000929
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JP 2001149085	A2	20010605	JP 2000-300778	20000929
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PRAI US 1999-156602P P 19990929

AB The present invention provides protein and DNA sequences of

\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* gene. The present  
invention further relates to novel apoprotein antigens encoded by  
\*\*\*mhp3\*\*\* gene for use in vaccines to prevent and treat diseases caused  
by infection with \*\*\*Mycoplasma\*\*\* hyopneumoniae. The invention  
further relates to method recombinant prodn. of such antigens.

TI Sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and  
therapeutic uses thereof

IN \*\*\*King, Kendall Wayne\*\*\* ; Madura, Rebecca Anne; Rosey, Everett Lee

AB The present invention provides protein and DNA sequences of

\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* gene. The present  
invention further relates to novel apoprotein antigens encoded by  
\*\*\*mhp3\*\*\* gene for use in vaccines to prevent and treat diseases caused  
by infection with \*\*\*Mycoplasma\*\*\* hyopneumoniae. The invention  
further relates to method recombinant prodn. of such antigens.

ST cDNA sequence \*\*\*Mycoplasma\*\*\* \*\*\*mhp3\*\*\* gene vaccine  
antimicrobial

IT Escherichia coli

(BL21 or Pz427; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Primers (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(DNA, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT \*\*\*Mycoplasma\*\*\* hyopneumoniae

(P46, P65, P97 and P102; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Immunostimulants

(adjuvants; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Proteins, specific or class

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(apoproteins, \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Eukaryote (Eukaryotae)

(as host cells; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Prokaryote

(as host; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Promoter (genetic element)

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(binding to \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Fusion proteins (chimeric proteins)

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(comprising \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT DNA

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(encoding \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Colorimetry

Nucleic acid hybridization

PCR (polymerase chain reaction)

(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Probes (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Test kits

(for diagnosis \*\*\*Mycoplasma\*\*\* infection; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Thioredoxins

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(fused with \*\*\*mhp3\*\*\* protein; sequences of \*\*\*Mycoplasma\*\*\*

hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Swine  
 (infected by \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
 uses thereof)

IT Antigens  
 Proteins, specific or class  
 RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU  
 (Biological study, unclassified); PRP (Properties); THU (Therapeutic use);  
 BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES  
 (Uses)  
 ( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
 antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Gene, microbial  
 RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP  
 (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU  
 (Occurrence); USES (Uses)  
 ( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
 antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Diagnosis  
 (mol.; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Genetic vectors  
 (pBAD/Thio-TOPO, expressing \*\*\*mhp3\*\*\* ; sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
 uses thereof)

IT DNA  
 RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
 (primer, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
 uses thereof)

IT Antibodies  
 RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
 (secondary, to pig; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
 antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Antimicrobial agents  
 DNA sequences  
 Molecular cloning  
 Protein sequences  
 (sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\*  
 and therapeutic uses thereof)

IT Mutagenesis  
 (site-directed, on antigen \*\*\*mhp3\*\*\* ; sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
 uses thereof)

IT Vaccines  
 (to \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Immunization  
 (vaccination; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Drugs  
 (veterinary; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334061-70-0P 334063-02-4P



RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)

(amino acid sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 9001-78-9, Alkaline phosphatase

RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 9003-99-0, Peroxidase

RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(horseradish, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334061-69-7 334062-98-5

RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)

(nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334073-41-5, 5: PN: EP1090995 SEQID: 5 unclaimed DNA 334073-43-7

334073-44-8 334073-45-9 334073-46-0 334073-47-1 334073-48-2

334073-49-3 334073-50-6 334073-51-7 334073-52-8 334073-53-9

334073-54-0 334073-55-1 334073-56-2 334073-57-3 334073-58-4

334073-59-5 334073-60-8 334073-61-9 334073-62-0 334073-63-1

334073-64-2 334073-65-3 334073-66-4 334073-67-5 334073-68-6

334073-69-7 334073-70-0 334073-71-1 334073-72-2 334073-73-3

RL: PRP (Properties)

(unclaimed nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334073-42-6 334073-74-4 334519-66-3

RL: PRP (Properties)

(unclaimed protein sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 183440-91-7 333997-53-8

RL: PRP (Properties)

(unclaimed sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

L3 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2002 ACS

AN 1996:225735 CAPLUS

DN 124:311404

TI Artificial transformation of mollicutes via polyethylene glycol- and electroporation-mediated methods

AU Dybvig, Kevin; Gasparich, Gail E.; \*\*\*King, Kendall W.\*\*\*

CS Department Comparative Medicine, University Alabama, Birmingham, AL, 35294, USA

SO Molecular and Diagnostic Procedures in Mycoplasmaology (1995), Volume 1, 179-84. Editor(s): Razin, Shmuel; Tully, Joseph G. Publisher: Academic, San Diego, Calif.

CODEN: 62QDA9

DT Conference; General Review

LA English

AB A review and discussion with 14 refs. about the methodol. and comparative efficiencies of the 2 title methods for transformation of mollicutes.

AU Dybvig, Kevin; Gasparich, Gail E.; \*\*\*King, Kendall W.\*\*\*

IT Electroporation

Mollicutes

\*\*\*Mycoplasma\*\*\*

Transformation, genetic

(artificial transformation of mollicutes by PEG- and electroporation-mediated methods)

L3 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2002 ACS

AN 1994:97439 CAPLUS

DN 120:97439

TI The development and use of \*\*\*mycoplasma\*\*\* cloning vectors

AU \*\*\*King, Kendall Wayne\*\*\*

CS Univ. Alabama, Birmingham, AL, USA

SO (1992) 114 pp. Avail.: Univ. Microfilms Int., Order No. DA9302477

From: Diss. Abstr. Int. B 1993, 53(9), 4482

DT Dissertation

LA English

AB Unavailable

TI The development and use of \*\*\*mycoplasma\*\*\* cloning vectors

AU \*\*\*King, Kendall Wayne\*\*\*

ST DNA cloning \*\*\*Mycoplasma\*\*\* vector construction

IT \*\*\*Mycoplasma\*\*\*

(DNA cloning in, vectors for, development and use of)

IT Genetic vectors

(for DNA cloning in \*\*\*Mycoplasma\*\*\*, development and use of)

IT Molecular cloning

(in \*\*\*Mycoplasma\*\*\*, vectors for, development and use of)

L3 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2002 ACS

AN 1993:248756 CAPLUS

DN 118:248756

TI Nucleotide sequence of \*\*\*Mycoplasma\*\*\* mycoides subspecies mycoides plasmid pKMK1

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin

CS Dep. Compar. Med., Univ. Alabama, Birmingham, AL, 35294, USA

SO Plasmid (1992), 28(1), 86-91

CODEN: PLSMDX; ISSN: 0147-619X

DT Journal

LA English

AB To facilitate the development of \*\*\*mycoplasma\*\*\* cloning vectors, the authors detd. the nucleotide sequence of pKMK1, a cryptic plasmid isolated from \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. It is 1875 bp in length and contains two open reading frames (ORFs) that share homol. with ORFs from members of a large family of gram-pos. bacterial plasmids which replicate via a single-stranded DNA intermediate. Putative origins of replication and candidate cloning sites have been identified.

TI Nucleotide sequence of \*\*\*Mycoplasma\*\*\* mycoides subspecies mycoides plasmid pKMK1

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin

AB To facilitate the development of \*\*\*mycoplasma\*\*\* cloning vectors,

the authors detd. the nucleotide sequence of pKMK1, a cryptic plasmid isolated from \*\*\*Mycoplasma\*\*\* mycoides subsp. mycoides. It is 1875 bp in length and contains two open reading frames (ORFs) that share homol. with ORFs from members of a large family of gram-pos. bacterial plasmids which replicate via a single-stranded DNA intermediate. Putative origins of replication and candidate cloning sites have been identified.

ST \*\*\*Mycoplasma\*\*\* plasmid pKMK1 sequence  
IT Gene, microbial  
RL: BIOL (Biological study)  
(for ORF1 and ORF2 proteins, of plasmid pKMK1 of \*\*\*Mycoplasma\*\*\* mycoides mycoides, nucleotide and encoded peptide sequences of)  
IT Deoxyribonucleic acid sequences  
(of plasmid pKMK1, of \*\*\*Mycoplasma\*\*\* mycoides mycoides)  
IT Protein sequences  
(of proteins ORF1 and ORF2, of plasmid pKMK1, of \*\*\*Mycoplasma\*\*\* mycoides mycoides)  
IT \*\*\*Mycoplasma\*\*\* mycoides mycoides  
(plasmid pKMK1 of, nucleotide and encoded peptide sequence of)  
IT Proteins, specific or class  
RL: PRP (Properties)  
(ORF 1, amino acid sequence of, of plasmid pKMK1 of \*\*\*Mycoplasma\*\*\* mycoides mycoides)  
IT Proteins, specific or class  
RL: PRP (Properties)  
(ORF 2, amino acid sequence of, of plasmid pKMK1 of \*\*\*Mycoplasma\*\*\* mycoides mycoides)  
IT Plasmid and Episome  
(pKMK1, of \*\*\*Mycoplasma\*\*\* mycoides mycoides, nucleotide sequence of)

L3 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2002 ACS

AN 1991:672567 CAPLUS

DN 115:272567

TI Plasmid transformation of \*\*\*Mycoplasma\*\*\* mycoides subspecies mycoides is promoted by high concentrations of polyethylene glycol

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin

CS Dep. Comp. Med., Univ. Alabama, Birmingham, AL, 35294, USA

SO Plasmid (1991), 26(2), 108-15

CODEN: PLSMDX; ISSN: 0147-619X

DT Journal

LA English

AB The recent isolation and characterization of 2 plasmids from M. mycoides subspecies mycoides has opened up new possibilities for studying \*\*\*mycoplasmal\*\*\* genetics. In order to facilitate the development of a genetic system in M. mycoides subsp. mycoides, parameters of polyethylene glycol (PEG)-mediated transformation were examd., as existing protocols prove very inefficient in this organism. The effects of PEG concn., DNA concn., presence of Ca<sup>2+</sup> ions, and choice of buffers on the transformation of the Tn916-contg. plasmid pAM120 into M. mycoides subsp. mycoides were examd. The stability of TN916 in the M. mycoides subsp. mycoides chromosome was also evaluated. The optimal PEG concn. (53-62% (w/v)) in the transformation mixt. was substantially higher than the PEG concn. reported to be optimal for transformation of other \*\*\*mycoplasmas\*\*\* (36% (w/v)). The PEG concns. used here were also higher than the concn. used to promote transformation or fusion of gram-pos. bacterial

protoplasts. A necessity for the presence of  $\text{Ca}^{2+}$  ions for optimal transformation was shown, as was the possible involvement of cell culture growth stage. The results demonstrate the need for expanding current transformation techniques for \*\*\*mycoplasmas\*\*\*. Once Tn916 inserts into the *M. mycoides* subsp. *mycoides* chromosome, it can transpose to other sites at a relatively high frequency.

TI Plasmid transformation of \*\*\*Mycoplasma\*\*\* mycoides subspecies mycoides is promoted by high concentrations of polyethylene glycol

AU \*\*\*King, Kendall W.\*\*\* ; Dybvig, Kevin

AB The recent isolation and characterization of 2 plasmids from *M. mycoides* subspecies mycoides has opened up new possibilities for studying \*\*\*mycoplasma\*\*\* genetics. In order to facilitate the development of a genetic system in *M. mycoides* subsp. *mycoides*, parameters of polyethylene glycol (PEG)-mediated transformation were examd., as existing protocols prove very inefficient in this organism. The effects of PEG concn., DNA concn., presence of  $\text{Ca}^{2+}$  ions, and choice of buffers on the transformation of the Tn916-contg. plasmid pAM120 into *M. mycoides* subsp. *mycoides* were examd. The stability of Tn916 in the *M. mycoides* subsp. *mycoides* chromosome was also evaluated. The optimal PEG concn. (53-62% (w/v)) in the transformation mixt. was substantially higher than the PEG concn. reported to be optimal for transformation of other \*\*\*mycoplasmas\*\*\* (36% (w/v)). The PEG concns. used here were also higher than the concn. used to promote transformation or fusion of gram-pos. bacterial protoplasts. A necessity for the presence of  $\text{Ca}^{2+}$  ions for optimal transformation was shown, as was the possible involvement of cell culture growth stage. The results demonstrate the need for expanding current transformation techniques for \*\*\*mycoplasmas\*\*\*. Once Tn916 inserts into the *M. mycoides* subsp. *mycoides* chromosome, it can transpose to other sites at a relatively high frequency.

ST \*\*\*Mycoplasma\*\*\* transformation polyethylene glycol

IT Transformation, genetic  
(of \*\*\*Mycoplasma\*\*\* mycoides mycoides, polyethylene glycol effect on)

IT 25322-68-3, PEG

RL: PRP (Properties)  
(genetic transformation of \*\*\*Mycoplasma\*\*\* mycoides mycoides enhanced by)

IT 7440-70-2, Calcium, biological studies

RL: BIOL (Biological study)  
( \*\*\*Mycoplasma\*\*\* mycoides mycoides genetic transformation efficiency in relation to)

L3 ANSWER 12 OF 12 JAPIO COPYRIGHT 2002 JPO

AN 2001-149085 JAPIO

TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae,  
PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN \*\*\*KING KENDALL WAYNE\*\*\* ; MADURA REBECCA ANNE; ROSEY EVERETT LEE

PA PFIZER PROD INC

PI JP 2001149085 A 20010605 Heisei

AI JP 2000-300778 (JP2000300778 Heisei) 20000929

PRAI US 1999-156602 19990929

SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 2001

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae  
\*\*\*mhp3\*\*\* gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by

\*\*\*mhp3\*\*\* used in vaccine for preventing and treating a disease caused by the infection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. A method for producing the antigen by the recombination is also provided.

COPYRIGHT: (C)2001,JPO

TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae, PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN \*\*\*KING KENDALL WAYNE\*\*\* ; MADURA REBECCA ANNE; ROSEY EVERETT LEE

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae \*\*\*mhp3\*\*\* gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by \*\*\*mhp3\*\*\* used in vaccine for preventing and treating a disease caused by the infection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. A method for producing the antigen by the recombination is also provided.

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=> e madura rebecca a/au

E1 2 MADURA PAUL/AU  
E2 25 MADURA R L/AU  
E3 0 --> MADURA REBECCA A/AU  
E4 1 MADURA REBECCA ANN/AU  
E5 3 MADURA REBECCA ANNE/AU  
E6 16 MADURA RICHARD L/AU  
E7 3 MADURA T/AU  
E8 1 MADURA TOMAS/AU  
E9 35 MADURAI S/AU  
E10 1 MADURAI S S/AU  
E11 2 MADURAM D M/AU  
E12 1 MADURAM D T/AU

=> s e3-e5

L4 4 ("MADURA REBECCA A"/AU OR "MADURA REBECCA ANN"/AU OR "MADURA REBECCA ANNE"/AU)

=> dup rem l4

PROCESSING COMPLETED FOR L4

L5 4 DUP REM L4 (0 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS

AN 2001:261139 CAPLUS

DN 134:294510

TI Sequences of Mycoplasma hyopneumoniae antigen mhp3 and therapeutic uses thereof

IN King, Kendall Wayne; \*\*\*Madura, Rebecca Anne\*\*\* ; Rosey, Everett Lee

PA Pfizer Products Inc., USA

SO Eur. Pat. Appl., 38 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

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PI EP 1090995 A2 20010411 EP 2000-308421 20000926

EP 1090995 A3 20010418

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO

BR 2000004488 A 20011113 BR 2000-4488 20000927

CN 1296953 A 20010530 CN 2000-129083 20000929

JP 2001149085 A2 20010605 JP 2000-300778 20000929

PRAI US 1999-156602P P 19990929

AB The present invention provides protein and DNA sequences of *Mycoplasma hyopneumoniae* antigen mhp3 gene. The present invention further relates to novel apoprotein antigens encoded by mhp3 gene for use in vaccines to prevent and treat diseases caused by infection with *Mycoplasma hyopneumoniae*. The invention further relates to method recombinant prodn. of such antigens.

L5 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS

AN 1999:708498 CAPLUS

DN 131:350240

TI sequence of *Neospora* protein antigens for vaccine development against neosporosis

IN Brake, David Alan; \*\*\*Madura, Rebecca Anne\*\*\*

PA Pfizer Products Inc., USA

SO Eur. Pat. Appl., 59 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

-----  
PI EP 953641 A2 19991103 EP 1999-301746 19990309

EP 953641 A3 20020313

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO

EP 1221485 A2 20020710 EP 2002-2959 19990309

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE,  
FI, CY

EP 1221486 A2 20020710 EP 2002-2960 19990309

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE,  
FI, CY

EP 1221487 A2 20020710 EP 2002-2961 19990309

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO, MK, CY, AL

JP 11332583 A2 19991207 JP 1999-81833 19990325

ZA 9902309 A 20001010 ZA 1999-2309 19990325

AU 9922437 A1 19991007 AU 1999-22437 19990326

CN 1232087 A 19991020 CN 1999-104381 19990326

BR 9902019 A 20000502 BR 1999-2019 19990326

PRAI US 1998-79389P P 19980326

US 1998-112282P P 19981215

EP 1999-301746 A3 19990309

AB The present invention provides isolated polynucleotide mols. comprising nucleotide sequences encoding GRA1, GRA2, SAG1, MIC1 and MAG1 antigen proteins from *Neospora caninum*, as well as recombinant vectors, transformed host cells, and recombinantly-expressed proteins. The present

invention further provides a polynucleotide mol. comprising the nucleotide sequence of the bidirectional GRA1/MAG1 promoter of *N. caninum*. The present invention further provides genetic constructs based on the polynucleotide mols. of the present invention that are useful in prepg. modified strains of *Neospora* cells for use in vaccines against neosporosis.

L5 ANSWER 3 OF 4 JAPIO COPYRIGHT 2002 JPO

AN 1999-332583 JAPIO

TI POLYNUCLEOTIDE MOLECULE ENCODING PROTEIN FROM NEOSPORA

IN BRAKE DAVID ALAN; \*\*\*MADURA REBECCA ANN\*\*\* ; DURTSCHI BECKY ANN;

KRISHNAN BALAKRISHNAN RAJENDRA; YODER SUSAN CHRISTINE

PA PFIZER PROD INC

PI JP 11332583 A 19991207 Heisei

AI JP 1999-81833 (JP11081833 Heisei) 19990325

PRAI US 1998-79389 19980326

US 1998-112282 19981215

SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1999

AB PROBLEM TO BE SOLVED: To obtain a new molecule having a nucleic acid sequence with a specific reading frame, containing a nucleic acid sequence encoding *Neospora* GRA1 protein, and useful for the production of vaccines against or as a diagnostic for *Neospora* as a cause of miscarriage, neonatal death or the like in mammals.

SOLUTION: This isolated new polynucleotide molecule is such one as to contain a nucleotide sequence encoding *Neospora* GRA1 protein, selected from the group consisting of nucleotide sequence with a reading frame (ORF) of about nt 205 to about nt 777 of formula I, nucleotide sequence with an ORF of about nt 605 to about nt 1,304 of formula II and nucleotide sequence with an ORF encoding GRA1 of plasmid pRC77 (ATCC 209685), being useful for the production of vaccines against or as a diagnostic for *Neospora* as the main cause of miscarriage, neonatal death, congenital infection and encephalitic diseases in mammals. This molecule is obtained by cloning the gene of GRA1 protein from the chromosomal DNA of *Neospora caninum*.

COPYRIGHT: (C)1999,JPO

L5 ANSWER 4 OF 4 JAPIO COPYRIGHT 2002 JPO

AN 2001-149085 JAPIO

TI NUCLEIC ACID OF mhp3 GENE OF *Mycoplasma hyopneumoniae*, PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN KING KENDALL WAYNE; \*\*\*MADURA REBECCA ANNE\*\*\* ; ROSEY EVERETT LEE

PA PFIZER PROD INC

PI JP 2001149085 A 20010605 Heisei

AI JP 2000-300778 (JP2000300778 Heisei) 20000929

PRAI US 1999-156602 19990929

SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 2001

AB PROBLEM TO BE SOLVED: To obtain *Mycoplasma hyopneumoniae* mhp3 gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by mhp3 used in vaccine for preventing and treating a disease caused by the infection of *Mycoplasma hyopneumoniae*. A method for producing the antigen by the recombination is also provided.

COPYRIGHT: (C)2001,JPO

=> e rosey everette l/au

E1 24 ROSEY EVERETT L/AU  
E2 10 ROSEY EVERETT LEE/AU  
E3 0 --> ROSEY EVERETTE L/AU  
E4 2 ROSEY J M/AU  
E5 1 ROSEY JOSEPH A/AU  
E6 1 ROSEY JOSEPH ALBERT/AU  
E7 8 ROSEY R/AU  
E8 7 ROSEY RICHARD/AU  
E9 7 ROSEY T/AU  
E10 1 ROSEYEAR P R/AU  
E11 6 ROSEZ A/AU  
E12 3 ROSEZIN H/AU

=> s e1-e2

L6 34 ("ROSEY EVERETT L"/AU OR "ROSEY EVERETT LEE"/AU)

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 25 DUP REM L6 (9 DUPLICATES REMOVED)

=> d l7 and (mycoplasm? or mhp?)

'AND' IS NOT A VALID FORMAT

'(MYCOPLASM?)' IS NOT A VALID FORMAT

'OR' IS NOT A VALID FORMAT

'MHP?') IS NOT A VALID FORMAT

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):d

'D' IS NOT A VALID FORMAT

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):bib

L7 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2002 ACS

AN 2002:368499 CAPLUS

DN 136:382847

TI Genes for antigenic proteins of Lawsonia and their use diagnosis and prophylaxis of Lawsonia infection

IN \*\*\*Rosey, Everett Lee\*\*\* ; King, Kendall Wayne; Good, Robert Trygve; Strugnell, Richard Anthony

PA Agriculture Victoria Services Pty. Ltd., Australia; Australian Pork Limited; Pfizer Products, Inc.

SO PCT Int. Appl., 155 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 2002038594	A1	20020516	WO 2001-AU1462 20011109
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W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,  
 UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,  
 DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,  
 BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG  
 AU 2002014810 A5 20020521 AU 2002-14810 20011109  
 PRAI AU 2000-1381 A 20001110  
 US 2000-249596P P 20001117  
 WO 2001-AU1462 W 20011109  
 RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD  
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s l7 and (mycoplasm? or mhp?)  
 L8 3 L7 AND (MYCOPLASM? OR MHP?)

=> d bib ab kwic 1-  
 YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L8 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1997:118765 BIOSIS

DN PREV199799425268

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's  
 vaccine potential.

AU King, Kendall W. (1); Faulds, Daryl H.; \*\*\*Rosey, Everett L.\*\*\* ;  
 Yancey, Robert J., Jr.

CS (1) 213 Schuring Road, Portage, MI 49024 USA

SO Vaccine, (1997) Vol. 15, No. 1, pp. 25-35.

ISSN: 0264-410X.

DT Article

LA English

AB The gene encoding \*\*\*Mhp1\*\*\* , a 124 kDa protein from  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
 product characterized. No significant homology to the gene or encoded  
 polypeptide was found in the Genbank, NBRF, or PIR databases, though this  
 protein appears similar to p97, a putative adhesin of M. hyopneumoniae  
 described by Zhang et al. (Infect. Immun. 63, 1013-1019, 1995). Two  
 repeated motifs were identified within the 3' end of the gene and encoded  
 polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
 S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
 expression and purification of the protein. Both the authentic and  
 recombinant proteins were recognized by sera from pigs infected with M.  
 hyopneumoniae. In an induced-disease model in pigs, coughing was reduced  
 in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\* , although  
 differences were not significant. Only minimal protection against lung  
 lesion formation was provided, and again differences between the  
 \*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's

vaccine potential.

AU King, Kendall W. (1); Faulds, Daryl H.; \*\*\*Rosey, Everett L.\*\*\* ;  
Yancey, Robert J., Jr.

AB The gene encoding \*\*\*Mhp1\*\*\* , a 124 kDa protein from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
product characterized. No significant homology to the gene or encoded  
polypeptide was found. . . Immun. 63, 1013-1019, 1995). Two repeated  
motifs were identified within the 3' end of the gene and encoded  
polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
expression and purification of the protein.. . . from pigs infected  
with M. hyopneumoniae. In an induced-disease model in pigs, coughing was  
reduced in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\* ,  
although differences were not significant. Only minimal protection against  
lung lesion formation was provided, and again differences between the  
\*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

BC \*\*\*Mycoplasmataceae\*\*\* 07512

Suidae \*85740

IT Sequence Data

amino acid sequence; nucleotide sequence

IT Miscellaneous Descriptors

BACTERIAL DISEASE; CHARACTERIZATION; \*\*\*MHP1\*\*\* GENE; \*\*\*MHP1\*\*\*  
PROTEIN; MOLECULAR GENETICS; \*\*\*MYCOPLASMA\*\*\* HYOPNEUMONIAE  
INFECTION; PHARMACOLOGY; VACCINE POTENTIAL

ORGN Super Taxa

Bacteria - General Unspecified: Eubacteria, Bacteria;  
\*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria; Suidae: Artiodactyla,  
Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name

bacteria (Bacteria - General Unspecified); pig (Suidae);  
\*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms

animals; artiodactyls; bacteria; chordates; eubacteria; mammals;  
microorganisms; nonhuman mammals; nonhuman vertebrates; vertebrates

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS

AN 2001:261139 CAPLUS

DN 134:294510

TI Sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and  
therapeutic uses thereof

IN King, Kendall Wayne; Madura, Rebecca Anne; \*\*\*Rosey, Everett Lee\*\*\*

PA Pfizer Products Inc., USA

SO Eur. Pat. Appl., 38 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI EP 1090995	A2	20010411	EP 2000-308421	20000926
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EP 1090995	A3	20010418		
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO

BR 2000004488	A	20011113	BR 2000-4488	20000927
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CN 1296953	A	20010530	CN 2000-129083	20000929
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JP 2001149085 A2 20010605 JP 2000-300778 20000929  
 PRAI US 1999-156602P P 19990929

AB The present invention provides protein and DNA sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* gene. The present  
 invention further relates to novel apoprotein antigens encoded by  
 \*\*\*mhp3\*\*\* gene for use in vaccines to prevent and treat diseases caused  
 by infection with \*\*\*Mycoplasma\*\*\* hyopneumoniae. The invention  
 further relates to method recombinant prodn. of such antigens.

TI Sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and  
 therapeutic uses thereof

IN King, Kendall Wayne; Madura, Rebecca Anne; \*\*\*Rosey, Everett Lee\*\*\*

AB The present invention provides protein and DNA sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* gene. The present  
 invention further relates to novel apoprotein antigens encoded by  
 \*\*\*mhp3\*\*\* gene for use in vaccines to prevent and treat diseases caused  
 by infection with \*\*\*Mycoplasma\*\*\* hyopneumoniae. The invention  
 further relates to method recombinant prodn. of such antigens.

ST cDNA sequence \*\*\*Mycoplasma\*\*\* \*\*\*mhp3\*\*\* gene vaccine  
 antimicrobial

IT Escherichia coli  
 (BL21 or Pz427; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Primers (nucleic acid)  
 RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
 (DNA, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
 uses thereof)

IT \*\*\*Mycoplasma\*\*\* hyopneumoniae  
 (P46, P65, P97 and P102; sequences of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Immunostimulants  
 (adjuvants; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Proteins, specific or class  
 RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified);  
 BIOL (Biological study); PREP (Preparation)  
 (apoproteins, \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Eukaryote (Eukaryotae)  
 (as host cells; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Prokaryote  
 (as host; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
 \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Promoter (genetic element)  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
 (Uses)  
 (binding to \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Fusion proteins (chimeric proteins)  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (comprising \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT DNA  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)

(encoding \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Colorimetry

Nucleic acid hybridization

PCR (polymerase chain reaction)

(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Probes (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Test kits

(for diagnosis \*\*\*Mycoplasma\*\*\* infection; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Thioredoxins

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(fused with \*\*\*mhp3\*\*\* protein; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Swine

(infected by \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Antigens

Proteins, specific or class

RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU  
(Biological study, unclassified); PRP (Properties); THU (Therapeutic use);  
BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES  
(Uses)

( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Gene, microbial

RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP  
(Properties); THU (Therapeutic use); BIOL (Biological study); OCCU  
(Occurrence); USES (Uses)

( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Diagnosis

(mol.; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Genetic vectors

(pBAD/Thio-TOPO, expressing \*\*\*mhp3\*\*\* ; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT DNA

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(primer, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Antibodies

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(secondary, to pig; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Antimicrobial agents  
DNA sequences  
Molecular cloning  
Protein sequences  
(sequence of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\*  
and therapeutic uses thereof)

IT Mutagenesis  
(site-directed, on antigen \*\*\*mhp3\*\*\* ; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT Vaccines  
(to \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Immunization  
(vaccination; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT Drugs  
(veterinary; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334061-70-0P 334063-02-4P  
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU  
(Biological study, unclassified); PRP (Properties); THU (Therapeutic use);  
BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES  
(Uses)  
(amino acid sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 9001-78-9, Alkaline phosphatase  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST  
(Analytical study); BIOL (Biological study); USES (Uses)  
(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)

IT 9003-99-0, Peroxidase  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST  
(Analytical study); BIOL (Biological study); USES (Uses)  
(horseradish, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae;  
sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\*  
and therapeutic uses thereof)

IT 334061-69-7 334062-98-5  
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP  
(Properties); THU (Therapeutic use); BIOL (Biological study); OCCU  
(Occurrence); USES (Uses)  
(nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334073-41-5, 5: PN: EP1090995 SEQID: 5 unclaimed DNA 334073-43-7  
334073-44-8 334073-45-9 334073-46-0 334073-47-1 334073-48-2  
334073-49-3 334073-50-6 334073-51-7 334073-52-8 334073-53-9  
334073-54-0 334073-55-1 334073-56-2 334073-57-3 334073-58-4  
334073-59-5 334073-60-8 334073-61-9 334073-62-0 334073-63-1  
334073-64-2 334073-65-3 334073-66-4 334073-67-5 334073-68-6  
334073-69-7 334073-70-0 334073-71-1 334073-72-2 334073-73-3  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334073-42-6 334073-74-4 334519-66-3

RL: PRP (Properties)

(unclaimed protein sequence; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 183440-91-7 333997-53-8

RL: PRP (Properties)

(unclaimed sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

L8 ANSWER 3 OF 3 JAPIO COPYRIGHT 2002 JPO

AN 2001-149085 JAPIO

TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae,  
PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN KING KENDALL WAYNE; MADURA REBECCA ANNE; \*\*\*ROSEY EVERETT LEE\*\*\*

PA PFIZER PROD INC

PI JP 2001149085 A 20010605 Heisei

AI JP 2000-300778 (JP2000300778 Heisei) 20000929

PRAI US 1999-156602 19990929

SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 2001

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae

\*\*\*mhp3\*\*\* gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by

\*\*\*mhp3\*\*\* used in vaccine for preventing and treating a disease caused

by the infection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. A method for

producing the antigen by the recombination is also provided.

COPYRIGHT: (C)2001,JPO

TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae,  
PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN KING KENDALL WAYNE; MADURA REBECCA ANNE; \*\*\*ROSEY EVERETT LEE\*\*\*

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae

\*\*\*mhp3\*\*\* gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by

\*\*\*mhp3\*\*\* used in vaccine for preventing and treating a disease caused

by the infection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. A method for

producing the antigen by the recombination is also provided.

COPYRIGHT: (C)2001,JPO

=> s mycoplasm? and mhp?

L9 58 MYCOPLASM? AND MHP?

=> dup rem l9

PROCESSING COMPLETED FOR L9

L10 26 DUP REM L9 (32 DUPLICATES REMOVED)

=> d bib ab kwic 1-

YOU HAVE REQUESTED DATA FROM 26 ANSWERS - CONTINUE? Y/(N):y

L10 ANSWER 1 OF 26 USPATFULL

AN 2002:165182 USPATFULL

TI Nucleic acids, proteins, and antibodies

IN Rosen, Craig A., Laytonsville, MD, UNITED STATES

Ruben, Steven M., Olney, MD, UNITED STATES

Barash, Steven C., Rockville, MD, UNITED STATES

PI US 2002086811 A1 20020704

AI US 2001-764861 A1 20010117 (9)

PRAI US 2000-179065P 20000131 (60)

US 2000-180628P 20000204 (60)  
US 2000-214886P 20000628 (60)  
US 2000-217487P 20000711 (60)  
US 2000-225758P 20000814 (60)  
US 2000-220963P 20000726 (60)  
US 2000-217496P 20000711 (60)  
US 2000-225447P 20000814 (60)  
US 2000-218290P 20000714 (60)  
US 2000-225757P 20000814 (60)  
US 2000-226868P 20000822 (60)  
US 2000-216647P 20000707 (60)  
US 2000-225267P 20000814 (60)  
US 2000-216880P 20000707 (60)  
US 2000-225270P 20000814 (60)  
US 2000-251869P 20001208 (60)  
US 2000-235834P 20000927 (60)  
US 2000-234274P 20000921 (60)  
US 2000-234223P 20000921 (60)  
US 2000-228924P 20000830 (60)  
US 2000-224518P 20000814 (60)  
US 2000-236369P 20000929 (60)  
US 2000-224519P 20000814 (60)  
US 2000-220964P 20000726 (60)  
US 2000-241809P 20001020 (60)  
US 2000-249299P 20001117 (60)  
US 2000-236327P 20000929 (60)  
US 2000-241785P 20001020 (60)  
US 2000-244617P 20001101 (60)  
US 2000-225268P 20000814 (60)  
US 2000-236368P 20000929 (60)  
US 2000-251856P 20001208 (60)  
US 2000-251868P 20001208 (60)  
US 2000-229344P 20000901 (60)  
US 2000-234997P 20000925 (60)  
US 2000-229343P 20000901 (60)  
US 2000-229345P 20000901 (60)  
US 2000-229287P 20000901 (60)  
US 2000-229513P 20000905 (60)  
US 2000-231413P 20000908 (60)  
US 2000-229509P 20000905 (60)  
US 2000-236367P 20000929 (60)  
US 2000-237039P 20001002 (60)  
US 2000-237038P 20001002 (60)  
US 2000-236370P 20000929 (60)  
US 2000-236802P 20001002 (60)  
US 2000-237037P 20001002 (60)  
US 2000-237040P 20001002 (60)  
US 2000-240960P 20001020 (60)  
US 2000-239935P 20001013 (60)

DT Utility

FS APPLICATION

LREP HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850

CLMN Number of Claims: 24

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 22023

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

SUMM . . . PF00653 90 244 393

2.1.1 Apoptosis domain

HBMGR22 933922 17 HMMER PFAM: FAD binding PF01494

60.3 360 545

2.1.1 domain

blastx.14 \*\*\*MhpA\*\*\* [Escherichia coli]

gi|1665746|dbj|BAA1 98% 309 542

3052.1|

85% 568 627

92% 545 583

HLTGA03 974851 18 HMMER PFAM: ICE-like protease PF00655.

SUMM . . . pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomonas* spp.), \*\*\**Mycoplasma*\*\*\* pneumoniae pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

SUMM . . . *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus* spp.; candidiasis, caused by *Candida*; and mucormycosis), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., \*\*\**Mycoplasma*\*\*\* and *Chlamydia* spp.), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and.

SUMM . . . urethra, including inflammatory disorders, such as balanoposthitis, balanitis xerotica obliterans, phimosis, paraphimosis, syphilis, herpes simplex virus, gonorrhea, non-gonococcal urethritis, chlamydia, \*\*\**mycoplasma*\*\*\*, trichomonas, HIV, AIDS, Reiter's syndrome, condyloma acuminatum, condyloma latum, and pearly penile papules; urethral abnormalities, such as hypospadias, epispadias, and.

SUMM . . . *Serratia*, *Yersinia*, *Shigella*), *Erysipelothrix*, *Haemophilus* (e.g., *Haemophilus influenzae* type B), *Helicobacter*, *Legionella* (e.g., *Legionella pneumophila*), *Leptospira*, *Listeria* (e.g., *Listeria monocytogenes*), \*\*\**Mycoplasma*\*\*\*, *Mycobacterium* (e.g., *Mycobacterium leprae* and *Mycobacterium tuberculosis*), *Vibrio* (e.g., *Vibrio cholerae*), *Neisseriaceae* (e.g., *Neisseria gonorrhea*, *Neisseria meningitidis*),



Pasteurellacea, Proteus, Pseudomonas. . .

L10 ANSWER 2 OF 26 USPATFULL

AN 2002:4155 USPATFULL

TI PAI-2 conjugates for the treatment and imaging of cancer

IN Ranson, Marie, Austinmer, AUSTRALIA

Allen, Barry John, Yowie Bay, AUSTRALIA

Bunn, Clive Leighton, West Ryde, AUSTRALIA

PI US 2002002134 A1 20020103

AI US 2001-790900 A1 20010223 (9)

PRAI AU 2000-5824 20000224

DT Utility

FS APPLICATION

LREP Stephen A. Bent, FOLEY & LARDNER, Washington Harbour, 3000 K Street,  
N.W., Suite 500, Washington, DC, 20007-5109

CLMN Number of Claims: 31

ECL Exemplary Claim: 1

DRWN 14 Drawing Page(s)

LN.CNT 1165

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method is described for detecting, visualizing, or treating cells,  
particularly cancerous cells, that express a uPA/uPAR complex. The  
method employs a PAI-2 conjugate molecule that comprises PAI-2 or a  
functional derivative, homologue, analogue, chemical equivalent or  
mimetic thereof, which PAI-2 is bound, linked, or otherwise associated  
with a toxin or label.

SUMM . . . Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L--methylalanine
Mala		
L--methylarginine	Marg	L--methylassparagine
Masn		
L--methylasspartate	Masp	L--methyl-t-butylglycine
Mtbug		
L--methylcysteine	Mcys	L-methylethylglycine
Metg		
L--methylglutamine	Mgln	L--methylglutamate
Mglu		
L--methylhistidine	Mhis	L--methylhomophenylalanine
***Mhphe***		
L--methylisoleucine	Mile	N-(2-methylthioethyl)glycine
Nmet		
L--methylleucine	Mleu	L--methyllysine
Mlys		
L--methylmethionine	Mmet	L--methylnorleucine
Mnle		
L--methylnorvaline	Mnva	L--methylornithine
Morn		
L--methylphenylalanine	Mphe	L--methylproline
Mpro		
L--methylserine	Mser	L--methylthreonine
Mthr		
L--methyltryptophan	Mtrp	L--methyltyrosine. . .

DETD [0111] Two million human breast cancer cells [MDA-MB-231 (  
\*\*\*mycoplasma\*\*\* free)] in sterile phosphate buffered saline (PBS)  
were injected sub-cutaneously into the mammary fat pad of first pair

breasts on. . .

DETD [0117] Two million human breast cancer cells [MDA-MB-231 (\*\*\*mycoplasma\*\*\* free)] in sterile phosphate buffered saline (PBS) were injected sub-cutaneously into the mammary fat pad of first pair breasts on. . .

L10 ANSWER 3 OF 26 CABA COPYRIGHT 2002 CABI

AN 2002:83102 CABA

DN 20023038045

TI The acute outbreak of Glasser's disease in a large pig farm

Ostry przypadek choroby Glassera w wielkotowarowej fermie swin

AU Pejsak, Z.; Zmudzki, J.; Walachowski, M.

CS Zaklad Chorob Swin Panstwowego Instytutu Weterynaryjnego, Al. Partyzantow 57, 24-100 Pulawy, Poland.

SO Medycyna Weterynaryjna, (2002) Vol. 58, No. 3, pp. 192-196. 18 ref.

ISSN: 0025-8628

DT Journal

LA Polish

SL English

AB Historically, Glasser's disease has been considered a sporadic disease of piglets. Presently, *Haemophilus parasuis*, the aetiological factor of this disease, is one of the serious problems associated with mixing swine from different herds and within large herds or with the introduction of new breeding stock into a herd. The role of concurrent infections in exacerbating of *H. parasuis* infections is problematic. The role of this pathogen in swine respiratory diseases is unclear. This report records an episode of severe *H. parasuis* infection in swine which was observed in a large pig farm. The infection proceeded in the herd as a serious, highly contagious enzootic with a high morbidity of 6- to 8-week-old weaned pigs. The observations were carried out in a large farm with 1800 sows and 50 000 piglets produced per year. The farrowing unit at the farm contained 30 farrowing pens. Every 3 days, about 330 piglets (24-27 days old) were weaned and moved to the nursery house where they stayed until aged 80 days, and after that moved to the fattening unit. The farm was PRRSV (porcine reproductive and respiratory syndrome virus), \*\*\*Mhp\*\*\* (\*\*\**Mycoplasma*\*\*\* *hyopneumoniae*), *Streptococcus suis* and App positive. In May 2001, the first clinical symptoms of Glasser's disease were observed among a group of piglets which were moved to the nursery house 3 weeks earlier. The signs of the disease were observed in about 25% of the pigs (approx equal to 80 pigs). The course of the disease was pre-acute or acute. The swine suddenly developed fever (40.5-41.5). During the next 3 months, the disease affected all consecutive groups of weaned pigs. A significant decrease of the number of affected pigs and number of losses was noticed 3 months after initial clinical symptoms. The main clinical symptoms of the disease were anorexia, cough, reluctance to rise, weight loss in all diseased pigs, and swollen joints in about 1/3 of sick pigs. During the first 3 months of the epizootic, 398 pigs died, with 15% of the weaners dying suddenly without premonitory signs of illness. Detailed postmortem examinations were performed on 55 pigs. Lesions were observed in all the pigs, which is typical of Glasser's disease. All pigs had serofibrinous pericarditis. Serofibrinous pleuritis and peritonitis were observed in the majority of the dead pigs. Approximately 60% of the pigs had pulmonary oedema and 25% had polyarthritis. Meningitis was observed in 5 necropsied animals. *H. parasuis* was isolated from the lungs and/or joints of 9 out of 30 of the bacteriologically investigated pigs. Colonies

appeared in the vicinity of the Staphylococcus aureus streak. The strain was non-haemolytic. In the primary culture, it consisted of Gram-negative, short rods. None of the sows or boars or piglets in the farrowing units were affected. The treatment of sick animals with amoxycillin gave less than satisfactory results. This is the first severe case of H. parasuis infection recorded in Poland, in which acute heavy losses among weaned pigs were observed.

AB . . . 80 days, and after that moved to the fattening unit. The farm was PRRSV (porcine reproductive and respiratory syndrome virus), \*\*\*Mhp\*\*\* ( \*\*\*Mycoplasma\*\*\* hyopneumoniae), Streptococcus suis and App positive. In May 2001, the first clinical symptoms of Glasser's disease were observed among a. . .

BT Haemophilus; Pasteurellaceae; Gracilicutes; bacteria; prokaryotes; \*\*\*Mycoplasma\*\*\* ; \*\*\*Mycoplasmataceae\*\*\* ; \*\*\*Mycoplasmatales\*\*\* ; Mollicutes; Tenericutes; Sus scrofa; Sus; Suidae; Suiformes; Artiodactyla; mammals; vertebrates; Chordata; animals; ungulates; Streptococcus; Streptococcaceae; Firmicutes

ORGN Haemophilus parasuis; \*\*\*Mycoplasma\*\*\* hyopneumoniae; pigs; Streptococcus suis

L10 ANSWER 4 OF 26 CAPLUS COPYRIGHT 2002 ACS

AN 2001:247540 CAPLUS

DN 134:276467

TI Method for detecting PCR amplified polynucleotides involving primer extension and fluorescent probes

IN Karpukhin, Alexander Vasilyevich; Veiko, Natalya Nikolaevna

PA Socrates Bio-Tech International Limited, Ire.; Lee, Nicholas, John

SO PCT Int. Appl., 22 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 2001023611	A2	20010405	WO 2000-GB3780	20001002
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WO 2001023611	A3	20011101		
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W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI GB 1999-23144 A 19990930

AB The present invention provides a method for detecting the amplification of a target polynucleotide. In the method, the target polynucleotide anneals to an oligonucleotide probe which incorporates a modified nucleotide (such as ethenodeoxyadenosine, ethenodeoxycytidine or 7-methyldeoxyguanosine) having a fluorescent characteristic which is modified by one or more neighboring unmodified nucleotides. An oligonucleotide primer annealed to the target polynucleotide is extended with a polymerase having a 5'-3' exonuclease activity, and the change in fluorescence is detected as the oligonucleotide probe is degraded by the exonuclease activity of the

polymerase, as the polymerase extends the primer and modification of the fluorescent characteristic of the modified nucleotide is reduced. The invention also provides a kit for detn. of amplified polynucleotides, which includes said materials. The claimed method, primers and modified probes contg. ethenodeoxyadenosine were used to detect \*\*\*Mycoplasma\*\*\* hominis, Salmonella typhimurium and human papillomavirus 16 PCR amplified DNA.

AB The present invention provides a method for detecting the amplification of a target polynucleotide. In the method, the target polynucleotide anneals to an oligonucleotide probe which incorporates a modified nucleotide (such as ethenodeoxyadenosine, ethenodeoxycytidine or 7-methyldeoxyguanosine) having a fluorescent characteristic which is modified by one or more neighboring unmodified nucleotides. An oligonucleotide primer annealed to the target polynucleotide is extended with a polymerase having a 5'-3' exonuclease activity, and the change in fluorescence is detected as the oligonucleotide probe is degraded by the exonuclease activity of the polymerase, as the polymerase extends the primer and modification of the fluorescent characteristic of the modified nucleotide is reduced. The invention also provides a kit for detn. of amplified polynucleotides, which includes said materials. The claimed method, primers and modified probes contg. ethenodeoxyadenosine were used to detect \*\*\*Mycoplasma\*\*\* hominis, Salmonella typhimurium and human papillomavirus 16 PCR amplified DNA.

IT \*\*\*Mycoplasma\*\*\* hominis  
(method for detecting PCR amplified polynucleotides involving primer extension and fluorescent probes, use of method in detection of amplified \*\*\*Mycoplasma\*\*\* hominis DNA in clin. samples)

IT 332434-71-6  
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)  
(16S rDNA specific ethenodeoxyadenosine-labeled probe \*\*\*Mhp1\*\*\* ; method for detecting PCR amplified polynucleotides involving primer extension and fluorescent probes, use of method in detection of amplified \*\*\*Mycoplasma\*\*\* hominis DNA in clin. samples)

IT 332434-70-5  
RL: ARG (Analytical reagent use); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)  
(16S rDNA specific ethenodeoxyadenosine-labeled probe \*\*\*Mhp2\*\*\* ; method for detecting PCR amplified polynucleotides involving primer extension and fluorescent probes, use of method in detection of amplified \*\*\*Mycoplasma\*\*\* hominis DNA in clin. samples)

L10 ANSWER 5 OF 26 CAPLUS COPYRIGHT 2002 ACS

AN 2001:261139 CAPLUS

DN 134:294510

TI Sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof

IN King, Kendall Wayne; Madura, Rebecca Anne; Rosey, Everett Lee

PA Pfizer Products Inc., USA

SO Eur. Pat. Appl., 38 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1090995	A2	20010411	EP 2000-308421	20000926
EP 1090995	A3	20010418		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 2000004488	A	20011113	BR 2000-4488	20000927
CN 1296953	A	20010530	CN 2000-129083	20000929
JP 2001149085	A2	20010605	JP 2000-300778	20000929
PRAI US 1999-156602P	P	19990929		
AB The present invention provides protein and DNA sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** gene. The present invention further relates to novel apoprotein antigens encoded by ***mhp3*** gene for use in vaccines to prevent and treat diseases caused by infection with ***Mycoplasma*** hyopneumoniae. The invention further relates to method recombinant prodn. of such antigens.				
TI Sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof				
AB The present invention provides protein and DNA sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** gene. The present invention further relates to novel apoprotein antigens encoded by ***mhp3*** gene for use in vaccines to prevent and treat diseases caused by infection with ***Mycoplasma*** hyopneumoniae. The invention further relates to method recombinant prodn. of such antigens.				
ST cDNA sequence ***Mycoplasma*** ***mhp3*** gene vaccine antimicrobial				
IT Escherichia coli (BL21 or Pz427; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Primers (nucleic acid) RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses) (DNA, for detecting ***Mycoplasma*** hyopneumoniae; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT ***Mycoplasma*** hyopneumoniae (P46, P65, P97 and P102; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Immunostimulants (adjuvants; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Proteins, specific or class RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation) (apoproteins, ***mhp3*** ; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Eukaryote (Eukaryotae) (as host cells; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Prokaryote (as host; sequences of ***Mycoplasma*** hyopneumoniae antigen ***mhp3*** and therapeutic uses thereof)				
IT Promoter (genetic element) RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)				

- (binding to \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Fusion proteins (chimeric proteins)  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(comprising \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT DNA  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(encoding \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Colorimetry  
Nucleic acid hybridization  
PCR (polymerase chain reaction)  
(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Probes (nucleic acid)  
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Test kits  
(for diagnosis \*\*\*Mycoplasma\*\*\* infection; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Thioredoxins  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(fused with \*\*\*mhp3\*\*\* protein; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Swine  
(infected by \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Antigens  
Proteins, specific or class  
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU  
(Biological study, unclassified); PRP (Properties); THU (Therapeutic use);  
BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES  
(Uses)  
( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Gene, microbial  
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP  
(Properties); THU (Therapeutic use); BIOL (Biological study); OCCU  
(Occurrence); USES (Uses)  
( \*\*\*mhp3\*\*\* ; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Diagnosis  
(mol.; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Genetic vectors  
(pBAD/Thio-TOPO, expressing \*\*\*mhp3\*\*\* ; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT DNA

- RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(primer, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Antibodies  
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(secondary, to pig; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Antimicrobial agents  
DNA sequences  
Molecular cloning  
Protein sequences  
(sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\*  
and therapeutic uses thereof)
- IT Mutagenesis  
(site-directed, on antigen \*\*\*mhp3\*\*\* ; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT Vaccines  
(to \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Immunization  
(vaccination; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT Drugs  
(veterinary; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen  
\*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT 334061-70-0P 334063-02-4P  
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU  
(Biological study, unclassified); PRP (Properties); THU (Therapeutic use);  
BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES  
(Uses)  
(amino acid sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT 9001-78-9, Alkaline phosphatase  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST  
(Analytical study); BIOL (Biological study); USES (Uses)  
(for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae; sequences of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic  
uses thereof)
- IT 9003-99-0, Peroxidase  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST  
(Analytical study); BIOL (Biological study); USES (Uses)  
(horseradish, for detecting \*\*\*Mycoplasma\*\*\* hyopneumoniae;  
sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae antigen \*\*\*mhp3\*\*\*  
and therapeutic uses thereof)
- IT 334061-69-7 334062-98-5  
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP  
(Properties); THU (Therapeutic use); BIOL (Biological study); OCCU  
(Occurrence); USES (Uses)  
(nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)
- IT 334073-41-5, 5: PN: EP1090995 SEQID: 5 unclaimed DNA 334073-43-7  
334073-44-8 334073-45-9 334073-46-0 334073-47-1 334073-48-2  
334073-49-3 334073-50-6 334073-51-7 334073-52-8 334073-53-9

334073-54-0 334073-55-1 334073-56-2 334073-57-3 334073-58-4  
334073-59-5 334073-60-8 334073-61-9 334073-62-0 334073-63-1  
334073-64-2 334073-65-3 334073-66-4 334073-67-5 334073-68-6  
334073-69-7 334073-70-0 334073-71-1 334073-72-2 334073-73-3

RL: PRP (Properties)

(unclaimed nucleotide sequence; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 334073-42-6 334073-74-4 334519-66-3

RL: PRP (Properties)

(unclaimed protein sequence; sequences of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

IT 183440-91-7 333997-53-8

RL: PRP (Properties)

(unclaimed sequence; sequences of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
antigen \*\*\*mhp3\*\*\* and therapeutic uses thereof)

L10 ANSWER 6 OF 26 USPATFULL

AN 2001:196806 USPATFULL

TI Simultaneous detection, identification and differentiation of  
eubacterial taxa using a hybridization assay

IN Jannes, Geert, Kessel-Lo, Belgium

Rossau, Rudi, Ekeren, Belgium

Van Heuverswyn, Hugo, Kalken, Belgium

PA Innogenetics N.V., Ghent, Belgium (non-U.S. corporation)

PI US 6312903 B1 20011106

AI US 1999-448894 19991129 (9)

RLI Division of Ser. No. US 1996-765332, filed on 23 Dec 1996, now patented,  
Pat. No. US 6025132

PRAI EP 1994-870106 19940624

EP 1995-870032 19950407

WO 1995-EP2452 19950623

DT Utility

FS GRANTED

EXNAM Primary Examiner: Horlick, Kenneth R.

LREP Nixon & Vanderhye P.C.

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN 103 Drawing Figure(s); 103 Drawing Page(s)

LN.CNT 3841

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method for detection and  
identification of at least one microorganism, or for the simultaneous  
detection of several microorganisms in a sample, involving the steps of:  
(i) if need be releasing, isolating or concentrating the polynucleic  
acids present in the sample; (ii) if need be amplifying the 16S-23S rRNA  
spacer region, or a part of it, with at least one suitable primer pair;  
(iii) hybridizing the polynucleic acids of step (i) or (ii) with at  
least one and preferably more than one of the spacer probes as mentioned  
in table Ia or equivalents of thereof, under the appropriate  
hybridization and wash conditions, and/or with a taxon-specific probe  
derived from any of the spacer sequences as represented in FIGS. 1-103  
under the same hybridization and wash conditions; (iv) detecting the  
hybrids formed in step (iii) with each of the probes used under  
appropriate hybridization and wash conditions; (v) identification of the  
microorganism(s) present in the sample from the differential



SUMM \*\*\*Mycoplasma\*\*\* species

SUMM It is also an aim of the present invention to provide probes or sets of probes for the detection of \*\*\*Mycoplasma\*\*\* strains, more particularly of *M. pneumoniae* and *M. genitalium*.

SUMM \*\*\*Mycoplasma\*\*\* pneumoniae

SUMM . . . 180)

MFO-ICG-2: ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)

MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

\*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 2: TGAATGTTCGTGGATGAACATTGATT (SEQ ID NO 35)

PA-ICG 3: CACTGGTGATCATTCAAGTCAAG (SEQ. . . ID NO 38)

MPN-ICG 1: ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

MGE-ICG 1: CACCCATTAATTTTTTCGGTGTAAAACCC (SEQ ID NO 51)

\*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)

STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)

STAU-ICG 3: AACGAAGCCGTATGTGAGCATTTGAC (SEQ. . . SUMM . . . 180)

MFO-ICG-2: ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)

MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

\*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 4: TGAATGTTCGT(G/A)(G/A)ATGAACATTGATITCTGGTC (SEQ ID NO 37)

PA-ICG 5: CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ. . . ID

NO

38)  
MPN-ICG 1: ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

49)  
MPN-ICG 2: CAGTTCTGAAAGAACATITCCGCTTCTTTC (SEQ ID NO 50)

50)  
MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

51)  
\*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

52)  
STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)

53)  
STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)

54)  
STAU-ICG 3: AACGAAGCCGTATGTGAGCATTGAC (SEQ. . .)

SUMM . . . (SEQ ID NO 1 to 33 and 175 to 191), of *Pseudomonas aeruginosa* (SEQ ID NO 34 to 38), of \*\*\*Mycoplasma\*\*\* species (SEQ ID NO 49 to 52), of *Staphylococcus aureus* (SEQ ID NO 53 to 56) and of *Acinetobacter baumannii*. . .

SUMM . . . ID NO 47)

CHTR-ICG 4: GAGTAGCGCGGTGAGCACGAGA (SEQ ID NO 201)

CHPS-ICG 1: GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)

MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

\*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

SUMM . . . the invention are designed for the detection of *Chlamydia* species (SEQ ID NO 45 to 48 and 201) and of \*\*\*Mycoplasma\*\*\* species (SEQ ID NO 51 and 52).

SUMM . . . 180)

MFO-ICG-2: ACTTGCGTGCGGATGCGGGAA (SEQ ID NO 181)

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCT (SEQ ID NO 188)

MML-TCG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

\*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

SUMM . . . 180)

MFO-ICG-2: ACTTGCGTGCGGATGCGGGAA (SEQ ID NO 181)

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCT (SEQ ID NO 188)

MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

\*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

SUMM The invention also provides for a method as described above to detect and identify one or more \*\*\*Mycoplasma\*\*\* strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

SUMM MPN-ICG 1: ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

\*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

SUMM and/or to any probe derived from SEQ ID NO 124 or 125 provided said probe hybridizes specifically with \*\*\*Mycoplasma\*\*\* species.

SUMM More particularly, the invention provides for a method as described above to detect and identify one or more \*\*\*Mycoplasma\*\*\* pneumoniae strains in a sample, wherein step (iii) comprises hybridizing to at

least one of the following probes:  
SUMM and/or to any probe derived from SEQ ID NO 125 provided said probe hybridizes specifically to \*\*\*Mycoplasma\*\*\* pneumoniae. According to a preferred embodiment, both these probes are used in combination.  
SUMM In another particular embodiment, the invention provides for a method as described above to detect and identify one or more \*\*\*Mycoplasma\*\*\* genitalium strains in a sample, wherein step (iii) comprises hybridizing to the following probe:  
SUMM and/or to any probe derived from SEQ ID NO 124 provided said probe hybridizes specifically to \*\*\*Mycoplasma\*\*\* genitalium.  
DRWD FIG. 49: represents the DNA sequence of the 16S-23S spacer region from \*\*\*Mycoplasma\*\*\* genitalium (U. Gobel) (SEQ ID NO 124)  
DRWD FIG. 50: represents the DNA sequence of the 16S-23S spacer region from \*\*\*Mycoplasma\*\*\* pneumoniae ATCC 29432 (SEQ ID NO 125)

DETD . . . 180

MFO-ICG-2	ACTTGGCGTGGGATGCGGGAA	181	
MKA-ICG-5	CCCTCAGGGATTTTCTGGGTGTTG	182	
MKA-ICG-6	GGACTCGTCCAAGAGTGTGTCC	183	
MKA-ICG-7	TCGGGCTTGGCCAGAGCTGTT	184	
MKA-ICG-8	GGGTGCGCAACAGCAAGCGA	185	
MKA-ICG-9	GATGCGTTGCCCCTACGGG	186	
MKA-ICG-10	CCCTACGGGTAGCGTGTCTTTTG	187	
MML-ICG-1	CGGATCGATTGAGTGCTTGTC	188	
MML-ICG-2	TCTAAATGAACGCACTGCCGATGG	189	
MCE-ICG-1	TGAGGGAGCCCGtGCCTGTA	190	
***MHP*** -ICG-1	CATGTTGGGCTTGATCGGGTGC	191	
PA-ICG 1	TGGTGTGCTGCGTGATCCGAT	34	
PA-ICG 2	TGAATGTTCGTGGATGAACATTGATT	35	
PA-ICG 3	CACTGGTGATCATTCAAGTCAAG	36	
PA-ICG 4	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	37	
PA-ICG 5	CTCTTTCACTGGTGATCATTCAAGTCAAG	38	
LIS-ICG 1	CAAGTAACCGAGAATCATCTGAAAGTGAATC	39	
LMO-ICG. . . 1	GGAAGAAGCCTGAGAAGGTTTCTGAC	45	
CHTR-ICG 2	GCATTTATATGTAAGAGCAAGCATTCTATTCA	46	
CHTR-ICG 3	GAGTAGCGTGGTGAGGACGAGA	47	
CHPS-ICG 1	GGATAACTGTCTTAGGACGGTTTGAC	48	
MPN-ICG 1	ATCGGTGGTAAATTAACCCAAATCCCTGT	49	
MPN-ICG 2	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	50	
MGE-ICG 1	CACCCATTAATTTTTTCGGTGTTAAACCC	51	
***Mycoplasma*** -ICG	CAAACTGAAAACGACAATCTTTCTAGTTCC	52	
STAU-ICG 1	TACCAAGCAAAACCGAGTGAATAAAGAGTT	53	
STAU-ICG 2	CAGAAGATGCGGAATAACGTGAC	54	
STAU-ICG 3	AACGAAGCCGTATGTGAGCATTGAC	55	
STAU-ICG 4	GAACGTAACCTCATGTTAACGTTTGACTTAT	56	
ACI-ICG 1	GCTTAAGTGCACAGTGCTCTAACTGA	57	
ACI-ICG 2	CACGGTAATTAGTGTGATCTGACGAAG	58	
BRU-ICG. . .			
DETD . . . Lactococcus casei	1	--	
Escherichia coli	1	--	
Hafnia halvei	1	--	
Agrobacterium tumefaciens	2	--	
***Mycoplasma*** dimorpha	1	--	
Clostridium tyrobutyricum	1	--	
Clostridium perfringens	1	--	
Clostridium sporogenes	1	--	

Clostridium. . .

DETD \*\*\*Mycoplasma\*\*\* pneumoniae and \*\*\*Mycoplasma\*\*\* genitalium

DETD \*\*\*Mycoplasmas\*\*\* are a group of the smallest prokaryotes known that are able to grow in cell-free media, lack a cell wall,. . .

DETD In humans, \*\*\*mycoplasmas\*\*\* have been recognized either as pathogenic organisms or as commensals. The best known pathogen is \*\*\*Mycoplasma\*\*\* pneumoniae, the causative agent of primary atypical pneumonia, especially in children and young adults. The diagnosis of M. pneumoniae has. . .

DETD Another pathogen, first isolated from urethral specimens from patients with nongonococcal urethritis, has been described as \*\*\*Mycoplasma\*\*\* genitalium. This \*\*\*mycoplasma\*\*\* has several properties in common with M. pneumoniae. Both species are pathogenic and both possess the capability to adhere to. . .

DETD . . . of both species is time-consuming and serology lacks specificity more rapid and more specific assays were developed to identify these \*\*\*mycoplasmas\*\*\*. The use of hybridization assays with DNA probes was described for these species, but despite good specificities these tests do. . .

DETD . . . species deposited in the EMBL databank (MPMAC and MGG37 respectively). Based on this information four probes were derived: one general \*\*\*Mycoplasma\*\*\* probe, two M. pneumoniae specific, and one M. genitalium specific probe:

DETD \*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC

DETD . . . 20% formamide at 50.degree. C.) to amplified spacer material from four M. pneumoniae strains, one M. genitalium strain and twenty-two non- \*\*\*Mycoplasma\*\*\* species strains. The general probe hybridized only to the five \*\*\*Mycoplasma\*\*\* strains tested, while the specific probes hybridized only to strains of the species for which they were designed.

DETD \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGTC

DETD The probes tested (MSI-ICG1, MXE-ICG-1, MFO-ICG-1, MFO-ICG-2, MML-ICG-1, MML-ICG-2, MCE-ICG-1 and \*\*\*MHP\*\*\* -ICG-1) specifically detected M. simiae M xenopi, M. fortuitum, M. malmoense, M. celatum and M. haemophilum respectively and showed no cross-hybridization. . .

DETD TABLE 8

additional mycobacterial probes

			MFO		MML				
	MUL	MGV	ICG-	MXE	ICG-1	MSI	ICG-1		
MCH	***MHP***								
species/type	strain	ICG-1	1	2	3	ICG-1	ICG-2	ICG-1	ICG-2
	ICG-1	ICG-1							

M.tuberculosis 8004 --- ----- . . .

DETD Buck G, O'Hara L, Summersgill J (1992). Rapid, sensitive detection of \*\*\*Mycoplasma\*\*\* pneumoniae in simulated clinical specimens by DNA amplification. Journal of Clinical Microbiology, 30: 3280-3283.

DETD . . . J, Angulo A, Van Zoest M, Quint W, Niesters H, Galama J, Melchers W (1992). Genus- and species-specific identification of \*\*\*mycoplasmas\*\*\* by 16S rRNA amplification. Applied and Environmental Microbiology, 58: 2606-2615.

L10 ANSWER 7 OF 26 USPATFULL

AN 2001:4264 USPATFULL

TI \*\*\*Mycoplasma\*\*\* recombinant polypeptides and vaccines

IN Browning, Glenn Francis, Parkville, Australia

Duffy, Michael Francis, Parkville, Australia  
Whithear, Kevin George, Parkville, Australia  
Walker, Ian Douglas, Parkville, Australia

PA The University of Melbourne, Parkville, Australia (non-U.S. corporation)

PI US 6171589 B1 20010109

WO 9721727 19970619

AI US 1998-91117 19980902 (9)

WO 1996-AU803 19961213

19980902 PCT 371 date

19980902 PCT 102(e) date

PRAI AU 1995-7127 19951213

DT Patent

FS Granted

EXNAM Primary Examiner: Minnifield, Nita; Assistant Examiner: Baskar, Padma

LREP Greenlee Winner and Sullivan PC

CLMN Number of Claims: 28

ECL Exemplary Claim: 1

DRWN 7 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 2343

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates generally to immunogens and their use in vaccine preparations. More particularly, the present invention is directed to a peptide or polypeptide or a derivative, homologue or analogue thereof which corresponds to, mimics, or cross-reacts with, B-cell or T-cell epitopes on surface polypeptides encoded by \*\*\*Mycoplasma\*\*\* pneumoniae or M. genitalium. The immunogens of the present invention are particularly useful in vaccine preparations for the prophylactic and therapeutic treatment of individuals against infections by \*\*\*Mycoplasma\*\*\* ssp. The present invention further provides diagnostic reagents for the detection of \*\*\*Mycoplasma\*\*\* ssp. in biological samples derived from individuals suspected of being infected therewith.

TI \*\*\*Mycoplasma\*\*\* recombinant polypeptides and vaccines

AB . . . homologue or analogue thereof which corresponds to, mimics, or cross-reacts with, B-cell or T-cell epitopes on surface polypeptides encoded by \*\*\*Mycoplasma\*\*\* pneumoniae or M. genitalium. The immunogens of the present invention are particularly useful in vaccine preparations for the prophylactic and therapeutic treatment of individuals against infections by \*\*\*Mycoplasma\*\*\* ssp. The present invention further provides diagnostic reagents for the detection of \*\*\*Mycoplasma\*\*\* ssp. in biological samples derived from individuals suspected of being infected therewith.

SUMM . . . derivative, homologue or analogue thereof which corresponds to, mimics, or cross-reacts with, B-cell or T-cell epitopes on polypeptides encoded by \*\*\*Mycoplasma\*\*\* pneumoniae and M. genitalium. Vaccine preparations comprising the peptides or polypeptides of the present invention are useful in protecting individuals against infections by species of the genus \*\*\*Mycoplasma\*\*\* .

SUMM The micro-organisms \*\*\*Mycoplasma\*\*\* pneumoniae is a pathogen of humans that typically colonises the upper respiratory tract.

\*\*\*Mycoplasma\*\*\* pneumoniae moves along the cilia of the respiratory epithelium until in close association with the host cell to which it .

SUMM The threat the M. pneumoniae poses to children and sensitized adults is considerable. \*\*\*Mycoplasma\*\*\* pneumoniae is the primary cause of

atypical pneumonia in young adults and children, although infected patients often present with symptoms. . .

SUMM . . . to infection. Accordingly, there is a clear need to develop agents useful in the diagnosis and prophylaxis of infection by \*\*\*mycoplasmas\*\*\*, in particular *M. pneumoniae*.

SUMM . . . polypeptides and derivatives, homologues or analogues thereof, provide the means to develop a range of diagnostic and prophylactic agents for \*\*\*Mycoplasma\*\*\* infection which were hitherto not available.

SUMM . . . isolated or recombinant polypeptide or a derivative, homologue or analogue thereof wherein said polypeptide is obtainable from a species of \*\*\*Mycoplasma\*\*\*.

SUMM . . . an isolated or recombinant polypeptide or derivative, homologue or analogue thereof wherein said polypeptide is obtainable from a species of \*\*\*Mycoplasma\*\*\* and has a predicted molecular weight of approximately 16 kDa.

SUMM . . . the isolated or recombinant polypeptide of the present invention or a derivative, homologue or analogue thereof is further obtainable from \*\*\*Mycoplasma\*\*\* pneumoniae or *M. genitalium* and *M. genitalium*.

SUMM (I) it is obtainable from \*\*\*Mycoplasma\*\*\* pneumoniae;

SUMM (I) it is obtainable from \*\*\*Mycoplasma\*\*\* genitalium;

SUMM . . . 4 are only 37.3% identical overall, as described in Example 21. Accordingly, the present invention further extends to any isolated \*\*\*Mycoplasma\*\*\* polypeptide which has properties of a surface polypeptide and is at least 35% identical to SEQ ID NO:1 or SEQ. . .

SUMM . . . provided an isolated polypeptide, or a derivative, homologue or analogue thereof wherein said polypeptide is obtainable from a species of \*\*\*Mycoplasma\*\*\* and wherein said polypeptide in its native form is a surface polypeptide which has adhesion properties.

SUMM . . . embodiment is directed to an isolated polypeptide, or a derivative, homologue or analogue thereof wherein said polypeptide is obtainable from \*\*\*Mycoplasma\*\*\* pneumoniae, has a molecular weight of approximately 110 kDa determined by SDS/PAGE, or a predicted molecular weight of approximately 116. . .

SUMM (I) it is obtainable from \*\*\*Mycoplasma\*\*\* pneumoniae;

SUMM . . . embodiment is directed to an isolated polypeptide, or a derivative, homologue or analogue thereof wherein said polypeptide is obtainable from \*\*\*Mycoplasma\*\*\* genitalium.

SUMM (I) it is obtainable from \*\*\*Mycoplasma\*\*\* genitalium;

SUMM The present invention extends to both isolated non-recombinant polypeptides, recombinant polypeptides and isolated recombinant polypeptides of \*\*\*Mycoplasma\*\*\* described in any of the foregoing embodiments.

SUMM . . . homologue or analogue thereof, wherein said polypeptide is produced in a bacterial cell or culture thereof belonging to the genus \*\*\*Mycoplasma\*\*\*, in particular a cell of *M. pneumoniae* or *M. genitalium* or a culture thereof or an *Escherichia coli* cell.

SUMM . . . do not alter the essential properties of said polypeptide, for example its immunogenicity or effectiveness as a peptide vaccine against \*\*\*Mycoplasma\*\*\* ssp, amongst others. Accordingly, a polypeptide may be isolated from a source in nature, or chemically synthesized. Furthermore, a polypeptide. . .

SUMM . . . context to refer to a polypeptide which is localised on, or intrinsically or extrinsically associated with, the surface layer of

\*\*\*Mycoplasma\*\*\* spp. and in particular M. pneumoniae or M. genitalium. A surface polypeptide, or at least an epitope thereof, is accessible. . .

SUMM . . . inherent properties of a non-recombinant polypeptide when it is present in the cell from which it originates, such as a

\*\*\*mycoplasma\*\*\* cell, in particular a M. pneumoniae cell. For example, the M. pneumoniae polypeptide set forth in SEQ ID NO:2 has. .

SUMM . . . a polypeptide component in a vaccine composition which is designed to protect an individual against infection by a species of

\*\*\*Mycoplasma\*\*\*, in particular to protect an individual against infection by \*\*\*Mycoplasma\*\*\* pneumoniae or M. genitalium. The essential feature of said polypeptide, or derivative, homologue or analogue thereof for the present purpose. . .

SUMM . . . or T-cell epitope of an isolated or recombinant polypeptide which in its native form is obtainable from a species of

\*\*\*Mycoplasma\*\*\* such as, but not limited to M. pneumoniae and has a predicted molecular weight of approximately 16 kDa or a. . .

SUMM . . . a homologue will still function as a peptide immunogen which mimics or cross-reacts to B-cell or T-cell epitopes of a

\*\*\*Mycoplasma\*\*\* spp. polypeptide of the present invention.

SUMM Furthermore, a homologue may be isolated or derived from the same or another \*\*\*Mycoplasma\*\*\* species. Preferred sources of homologues of

a \*\*\*Mycoplasma\*\*\* pneumoniae polypeptide according to the present invention are M. genitalium, M. penetrans, M. iowae, M. gallisepticum, M. imitans, M. muris,. . .

SUMM . . . L-.alpha.-methylalanine Mala

L-.alpha.-methylarginine Marg L-.alpha.-methyl- Masn  
asparagine

L-.alpha.-methylaspartate Masp L-.alpha.-methyl-t- Mtbug  
butylglycine

L-.alpha.-methylcysteine Mcys L-methylethyl- Metg  
glycine

L-.alpha.-methylglutamine Mglu L-.alpha.-methyl- Mglu  
glutamate

L-.alpha.-methylhistidine Mhis L-.alpha.-methylhomo \*\*\*Mhphe\*\*\*  
phenylalanine

L-.alpha.-methylisoleucine Mile N-(2-methylthio- Nmet  
ethyl) glycine

L-.alpha.-methylleucine Mleu L-.alpha.-methyllysine Mlys

L-.alpha.-methylmethionine Mmet L-.alpha.-methyl- Mnle  
norleucine

L-.alpha.-methylnorvaline Mnva L-.alpha.-methylornithine Morn

L-.alpha.-methylphenylalanine Mphe L-.alpha.-methylproline Mpro

L-.alpha.-methylserine Mser. . .

SUMM . . . embodiment of the invention, the derivative is useful as an immunogen to elicit the production of antibodies capable of recognising \*\*\*Mycoplasma\*\*\* pneumoniae or at least a protein component thereof. According to this embodiment, there is provided a recombinant polypeptide derivative of. . .

SUMM . . . in said mammal. More preferably, the immunogen of the present invention when administered to a mammal, induces humoral immunity against \*\*\*Mycoplasma\*\*\* spp. in particular M. pneumoniae or M. genitalium in said primate. Still more preferably, the immunogen when administered, prevents the onset, development or progression, of

symptoms associated with \*\*\*Mycoplasma\*\*\* pneumoniae infections, for example atypical pneumonia, or lung lesions, or inflammation of the respiratory tract, or inflammation of the central. . .

SUMM . . . a vaccine composition comprising a polypeptide component which comprises an isolated immunogenic polypeptide obtainable or derived from a species of \*\*\*Mycoplasma\*\*\*, or alternatively, a recombinant immunogenic polypeptide comprising an amino acid sequence similar or identical to said isolated immunogenic polypeptide, in. . .

SUMM . . . when ingested, injected, or otherwise administered to a mammal. In a preferred embodiment, said vaccine induces humoral immunity against a \*\*\*Mycoplasma\*\*\* spp., in particular M. pneumoniae or M. genitalium, when injected, or otherwise administered to a mammal. More preferably, said vaccine. . .

SUMM . . . present invention provides a vaccine composition comprising an isolated or recombinant immunogenic polypeptide which is obtainable from a species of \*\*\*Mycoplasma\*\*\*, in combination with a pharmaceutically acceptable carrier or diluent, wherein said polypeptide is further characterised by any of the following. . .

SUMM According to this aspect of the invention, said vaccine mediates an immune response against \*\*\*Mycoplasma\*\*\* spp., in particular M. pneumoniae or M. genitalium, when the vaccine is injected, or otherwise administered to a mammal, for. . .

SUMM . . . fluorescent tag, the only requirement being that said immunologically interactive molecule is capable of binding to a polypeptide obtainable from \*\*\*Mycoplasma\*\*\* spp., or a derivative, homologue or analogue thereof, or to a molecule which mimics the 3-dimensional structure of same. In. . .

SUMM . . . specifically reactive with a polypeptide which comprises, mimics, or cross-reacts with a B cell or T cell epitope of a \*\*\*Mycoplasma\*\*\* polypeptide according to the embodiments described herein, in particular a polypeptide set forth in SEQ ID NO:1 or SEQ ID.

SUMM Accordingly, a further aspect of the present invention contemplates a method of detecting a polypeptide of \*\*\*Mycoplasma\*\*\* spp., in particular a polypeptide of M. pneumoniae or M. genitalium in serum, mucus, tissue extract, or other biological fluid. . .

SUMM Accordingly, the present invention also contemplates a kit of the rapid and convenient assay for a polypeptide of \*\*\*Mycoplasma\*\*\* spp., in particular a polypeptide of M. pneumoniae or M. genitalium in serum, mucus, tissue extract, or other biological fluid.

SUMM . . . aware that the subject kit is also useful for the purpose of determining the presence of whole cells of said \*\*\*Mycoplasma\*\*\* ssp.

SUMM A further aspect of the present invention provides a method of assaying for the presence of antibodies against a \*\*\*Mycoplasma\*\*\* ssp. in a mammal such as a human, said method comprising contacting a biological sample from said mammal with an. . .

SUMM . . . are capable of binding to one or more epitopes which in their native state are localised on the surface of \*\*\*Mycoplasma\*\*\* spp. in particular M. pneumoniae or M. genitalium for example a surface epitope of the surface polypeptide which is set. . .

SUMM In a more preferred embodiment, the present invention provides a method of assaying for the presence of antibodies against a \*\*\*Mycoplasma\*\*\* ssp. in a human individual, said method comprising contacting a biological sample obtained from said individual with an isolated or. . .



SUMM . . . will usually have arisen as a result of infection of the individual from whom the biological sample is derived by \*\*\*Mycoplasma\*\*\* spp., in particular *M. pneumoniae* or *M. genitalium*.

SUMM . . . for the present purposes, the only requirement being that said biological sample contains sufficient antibodies against a surface polypeptide of \*\*\*Mycoplasma\*\*\* spp., in particular a surface polypeptide of *M. pneumoniae* or *M. genitalium*, to enable the detection of the antibody-antigen complex.

SUMM . . . said mammal has been, at the time a biological sample was taken, infected with a microorganism belonging to the genus \*\*\*Mycoplasma\*\*\*, in particular *M. pneumoniae* such that antibodies to said microorganism have been produced in response to infection.

SUMM Accordingly, an alternative embodiment of the present invention provides a method of detection of \*\*\*Mycoplasma\*\*\* infection in an individual, said method comprising contacting a biological sample obtained from said individual with an isolated or recombinant. . .

SUMM . . . invention, the recombinant polypeptide is immobilised on a solid substrate and the biological sample containing antibodies against a polypeptide of \*\*\*Mycoplasma\*\*\*, in particular *M. pneumoniae* or *M. genitalium*, is brought into contact with the bound antigen. After a suitable period of. . .

SUMM Accordingly, the present invention also contemplates a kit for the rapid and convenient assay of infection by \*\*\*Mycoplasma\*\*\* spp., in particular *M. pneumoniae* in an individual comprising, in a first compartment several first containers adapted to contain the. . .

SUMM . . . positive result for comparison to the results obtained for the test sample. A positive result is indicative of infection by \*\*\*Mycoplasma\*\*\* spp., in particular *M. pneumoniae* or *M. genitalium*.

SUMM . . . acid molecule comprising a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a \*\*\*Mycoplasma\*\*\* spp. polypeptide according to any of the embodiments hereinbefore described.

SUMM . . . molecule which encodes a polypeptide which comprises, mimics, or cross-reacts with a B cell or T cell epitope of a \*\*\*Mycoplasma\*\*\* spp. polypeptide according to any of the embodiments hereinbefore described.

SUMM (i) encodes or is complementary to a sequence which encodes a \*\*\*Mycoplasma\*\*\* spp. polypeptide with a predicted molecular weight of approximately 16 kDa or 116 kDa, preferably the *M. pneumoniae* polypeptide set. . .

SUMM The genetic sequences which encodes a \*\*\*Mycoplasma\*\*\* spp. polypeptide according to any of the embodiments hereinbefore described, in particular a genetic sequence which encodes or is complementary. . . may differ by one or more nucleotide substitutions, deletions and/or additions. Accordingly, the present invention extends to genes encoding said \*\*\*Mycoplasma\*\*\* polypeptides or derivatives, homologues or analogues thereof, or nucleic acid molecules which are at least useful as genetic probes, or. . .

SUMM . . . embodiment, the genetic sequences of the present invention are employed to identify and isolate similar genes, form any species of \*\*\*Mycoplasma\*\*\*, for example *M. pneumoniae*, *M. genitalium*, or *M. gallisepticum* amongst others, and from other organisms.

SUMM . . . fungal cell, insect cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from a species of

\*\*\*Mycoplasma\*\*\*, in particular *M. pneumoniae*, *M. gallisepticum*, *M. pen-  
trans*, *M. iowae*, *M. muris*, *M. urealyticum*, *M. pirum*, *M. imitans* or  
*M.* . . .

SUMM . . . of at least 10 nucleotides in length to a nucleic acid  
"template molecules", said template molecule herein defined as a "

\*\*\*mycoplasma\*\*\* immunogen genetic sequence", "nycoplasma-like  
immunogen genetic sequence", or a functional part thereof, or its  
complementary sequence. Specific nucleic acid molecule. . .

SUMM . . . of the present invention, the nucleic acid primer molecules are  
derived from opposite DNA strands of a genetic sequence of  
\*\*\*Mycoplasma\*\*\* sp., in particular *M. pneumoniae*, which encodes a  
polypeptide according to any of the embodiments hereinbefore described.  
Preferably, the nucleic. . .

SUMM The \*\*\*mycoplasma\*\*\* immunogen genetic sequence or  
\*\*\*mycoplasma\*\*\* -like immunogen genetic sequence may be in a  
recombinant form, in a bacterial cell, virus particle, bacteriophage  
particle, fungal cell, yeast cell, insect cell, animal cell, or a plant  
cell. Preferably, the related genetic sequence originates from  
\*\*\*Mycoplasma\*\*\* spp., for example *M. pneumoniae*, *M. genitalium*, *M.*  
*pen-  
trans*, *M. iowae*, *M. muris*, *M. urealyticum*, *M. pirum*, *M. imitans* or.  
. . .

SUMM The present invention extends to the detection of a nucleic acid  
molecule which encodes a polypeptide of \*\*\*Mycoplasma\*\*\* spp., in  
particular a polypeptide of *M. pneumoniae*, wherein said polypeptide is  
according to any of the embodiments hereinbefore described. . .  
complement, or a derivative, homologue or analogue thereof. Accordingly,  
said method is useful for the purpose of detecting the micro-organism  
\*\*\*Mycoplasma\*\*\* spp., in particular *M. pneumoniae* in said serum,  
mucus, tissue extract, or biological fluid.

SUMM The present invention clearly contemplates a kit for the rapid detection  
of the micro-organism \*\*\*Mycoplasma\*\*\* spp., in particular *M.*  
*pneumoniae* in a biological sample, said kit being compartmentalized to  
contain in a first compartment, one. . . or more nucleic acid  
molecules which encode, or are complementary to a nucleic acid molecule  
which encodes a polypeptide of \*\*\*Mycoplasma\*\*\* spp., in particular  
a polypeptide of *M. pneumoniae* as hereinbefore described in embodiment.  
In a particularly preferred embodiment, the first. . .

SUMM . . . a genetic construct comprising a sequence of nucleotides which  
encodes, or is complementary to a nucleotide sequence which encodes a  
\*\*\*Mycoplasma\*\*\* spp. polypeptide as hereinbefore described in any  
embodiment, in particular the *M. pneumoniae* polypeptide set forth in SEQ  
ID NO:1. . .

SUMM . . . B cell or T cell epitopes which mimic, or cross-react, with a B  
cell or T cell epitope of a \*\*\*Mycoplasma\*\*\* spp. polypeptide as  
hereinbefore described in any embodiment, in particular the *M.*  
*pneumoniae* polypeptide set forth in SEQ ID NO:1. . .

DRWD . . . detergent phase polypeptides; lane 3, Triton X-114 aqueous  
phase polypeptides; lane 4, whole cell proteins. Arrows indicate the 110  
kDa \*\*\*Mycoplasma\*\*\* polypeptide (upper arrow) and a 70 kDa  
\*\*\*Mycoplasma\*\*\* polypeptide (lower arrow) enriched in the detergent  
phase.

DRWD . . . of a Southern blot showing the presence of homologues of the  
EcoRI fragment of *M. pneumoniae* in other species of \*\*\*Mycoplasma\*\*\*  
. Species names are indicated at the top of each lane. Fragment lengths  
(bp) are indicated on the left of the. . .

DETD Reference herein to \*\*\*Mycoplasma\*\*\* shall be taken to refer to M. pneumoniae strain FH grown in SP4 medium in glass bottles at 37.degree. C.

DETD Triton X-114 (Tx-114) partitioning adapted from the method of Bordier (1981) was used to isolate amphiphilic \*\*\*Mycoplasma\*\*\* proteins in the detergent phase. Triton X-114 was precondensed three times with PBS. The culture of M. pneumoniae in a. . .

DETD . . . agarose gel and transferred to nylon membrane (Amersham Hybond N.sup.-) as per the manufacturers instructions. Digested genomic DNA from these \*\*\*Mycoplasma\*\*\* spp. was hybridised to each of the labelled clones as described by Sambrook et al. (1989) after the method of. . .

DETD Other closely related \*\*\*mycoplasma\*\*\* lack homologous genes

DETD 15. Koskiniemi, M (1993). CNA manifestations associated with \*\*\*Mycoplasma\*\*\* pneumoniae infections: summary of cases at the University of Helsinki and review. Clin Infect Dis.

DETD 27. Zagami, A. S. et al, Detection of \*\*\*Mycoplasma\*\*\* pneumoniae in CSF of a patient with encephalitis. In: Australia Society for Microbiology Annual Scientific Meeting, 1994, Melbourne, Victoria, Australia:. . .

CLM What is claimed is:

1. An isolated or recombinant immunogenic polypeptide of a \*\*\*Mycoplasma\*\*\* ssp. having a molecular weight selected from the group consisting of: (i) a predicted molecular weight as determined from the. . .

2. The isolated or recombinant immunogenic polypeptide according to claim 1 wherein said polypeptide is isolated from \*\*\*Mycoplasma\*\*\* pneumoniae or wherein said polypeptide is isolated from a recombinant bacterial cell expressing said polypeptide.

7. The isolated or recombinant immunogenic polypeptide according to claim 1 derived from \*\*\*Mycoplasma\*\*\* ssp. selected from the group consisting of M. penetrans, M. iowae, M. gallisepticum, M. genitalium, M. imitans, M. muris, M.. . .

. . . isolated or recombinant immunogenic polypeptide according to claim 1 wherein the bacterial cell is an Escherichia coli cell or a \*\*\*Mycoplasma\*\*\* ssp. cell.

15. A vaccine composition for the therapeutic or prophylactic treatment of a mammalian subject against infection by a \*\*\*Mycoplasma\*\*\* ssp., said composition comprising the isolated or recombinant immunogenic polypeptide according to claim 1 in an amount sufficient to mediate. .

18. The vaccine composition according to claim 15 wherein the \*\*\*Mycoplasma\*\*\* ssp. is M. pneumoniae.

. . . 19. The vaccine composition according to claim 15 wherein said vaccine composition is capable of inducing humoral immunity against said \*\*\*Mycoplasma\*\*\* ssp.

27. An isolated or recombinant immunogenic polypeptide of \*\*\*Mycoplasma\*\*\* ssp. having a molecular weight selected from the group consisting of: (i) a predicted molecular weight as determined from the. . . determined using SDS/PAGE wherein said isolated or recombinant polypeptide is detected by a process comprising: (i)

hybridizing nucleic acid of \*\*\*Mycoplasma\*\*\* spp. to a probe or primer comprising the nucleotide sequence set forth in SEQ ID NO:3 under at least low. . .

28. An isolated or recombinant immunogenic polypeptide of \*\*\*Mycoplasma\*\*\* spp. having a molecular weight selected from the group consisting of: (i) a predicted molecular weight as determined from the. . . as determined using SDS/PAGE wherein said isolated or recombinant polypeptide is detected by a process comprising contacting protein of said \*\*\*Mycoplasma\*\*\* with an antibody molecule that binds specifically to the isolated or recombinant protein of claim 1.

L10 ANSWER 8 OF 26 USPATFULL

AN 2000:21384 USPATFULL

TI Methods and compositions for enhancing sensitivity in the analysis of biological-based assays

IN Ness, Jeffrey Van, Seattle, WA, United States

Tabone, John C., Bothell, WA, United States

Howbert, J. Jeffry, Bellevue, WA, United States

Mulligan, John T., Seattle, WA, United States

PA Rapigene, Inc., Bothell, WA, United States (U.S. corporation)

PI US 6027890 20000222

AI US 1997-898501 19970722 (8)

RLI Continuation-in-part of Ser. No. US 1997-787521, filed on 22 Jan 1997, now abandoned

PRAI US 1996-10436P 19960123 (60)

US 1996-15402P 19960321 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Houtteman, Scott W.

LREP Seed and Berry LLP

CLMN Number of Claims: 72

ECL Exemplary Claim: 1

DRWN 19 Drawing Figure(s); 19 Drawing Page(s)

LN.CNT 6333

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods are provided for detecting the binding of a first member to a second member of a ligand pair, comprising the steps of (a) combining a set of first tagged members with a biological sample which may contain one or more second members, under conditions, and for a time sufficient to permit binding of a first member to a second member, wherein said tag is correlative with a particular first member and detectable by non-fluorescent spectrometry, or potentiometry, (b) separating bound first and second members from unbound members, (c) cleaving the tag from the tagged first member, and (d) detecting the tag by non-fluorescent spectrometry, or potentiometry, and therefrom detecting the binding of the first member to the second member.

DETD . . . (5HIAA), 3-hydroxykynurenine, 3-hydroxymandelic acid, 3-hydroxy-4-methoxyphenylethylamine, 4-hydroxyphenylacetic acid (4HPAC), 4-hydroxyphenyllactic acid (4HPLA), 5-hydroxytryptophan (5HTP), 5-hydroxytryptophol (5HTOL), 5-hydroxytryptamine (5HT), 5-hydroxytryptamine sulfate, 3-methoxy-4-hydroxyphenylglycol ( \*\*\*MHPG\*\*\* ), 5-methoxytryptamine, 5-methoxytryptophan, 5-methoxytryptophol, 3-methoxytyramine (3MT), 3-methoxytyrosine (3-OM-DOPA), 5-methylcysteine, 3-methylguanine, bufotenin, dopamine

dopamine-3-glucuronide, dopamine-3-sulfate, dopamine-4-sulfate, epinephrine, epinine, folic acid, glutathione (reduced),. . .  
DETD . . . thus applicable to tissue culture cells, animal cells, animal tissue, blood cells (e.g., reticulocytes, lymphocytes), plant cells, bacteria, yeasts, viruses, \*\*\*mycoplasmas\*\*\* , protozoa, fungi and the like. By detecting a specific hybridization between nucleic acid probes of a known source, the specific. . .

L10 ANSWER 9 OF 26 USPTFULL

AN 2000:18218 USPTFULL

TI Probes targeted to rRNA spacer regions, methods and kits for using said probes, for the detection of respiratory tract pathogens

IN Jannes, Geert, Kessel-Lo, Belgium

Rossau, Rudi, Ekeren, Belgium

Van Heuverswyn, Hugo, Kalken, Belgium

PA Innogenetics N.V., Ghent, Belgium (non-U.S. corporation)

PI US 6025132 20000215

WO 9600298 19960104

AI US 1996-765332 19961223 (8)

WO 1995-EP2452 19950623

19961223 PCT 371 date

19961223 PCT 102(e) date

PRAI EP 1994-870106 19940624

EP 1995-870032 19950407

DT Utility

FS Granted

EXNAM Primary Examiner: Horlick, Kenneth R.

LREP Nixon & Vanderhye P.C.

CLMN Number of Claims: 32

ECL Exemplary Claim: 1

DRWN 103 Drawing Figure(s); 103 Drawing Page(s)

LN.CNT 6360

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method for detection and identification of at least one microorganism, or for the simultaneous detection of several microorganisms in a sample, comprising the steps of: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, with at least one suitable primer pair, (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least one and preferably more than one of the spacer probes as mentioned in table 1a or equivalents of thereof, under the appropriate hybridization and wash conditions, and/or with a taxon-specific probe derived from any of the spacer sequences as represented in FIGS. 1-103 under the same hybridization and wash conditions; (iv) detecting the hybrids formed in step (iii) with each of the probes used under appropriate hybridization and wash conditions; (v) identification of the microorganism(s) present in the sample from the differential hybridization signals obtained in step (iv).

SUMM \*\*\*Mycoplasma\*\*\* species

SUMM It is also an aim of the present invention to provide probes or sets of probes for the detection of \*\*\*Mycoplasma\*\*\* strains, more particularly of M. pneumoniae and M. genitalium.

SUMM \*\*\*Mycoplasma\*\*\* pneumoniae

SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC

(SEQ ID NO 191)  
SUMM \*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC  
(SEQ ID NO 52)  
SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC  
(SEQ ID NO 191)  
SUMM \*\*\*Mycoplasma\*\*\* -ICG : CAAAACTGAAAACGACAATCTTTCTAGTTCC  
(SEQ ID NO 52)  
SUMM . . . (SEQ ID NO 1 to 33 and 175 to 191, of *Pseudomonas aeruginosa*  
(SEQ ID NO 34 to 38), of \*\*\*Mycoplasma\*\*\* species (SEQ ID NO, 49 to  
52), of *Staphylococcus aureus* (SEQ ID NO 53 to 56) and of *Acinetobacter*  
*baumanii*. . .  
SUMM \*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC  
(SEQ ID NO 52)  
SUMM . . . the invention are designed for the detection of *Chlamydia*  
species (SEQ ID NO 45 to 48 and 201) and of \*\*\*Mycoplasma\*\*\* species  
(SEQ ID NO 51 and 52).  
SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC  
(SEQ ID NO 191)  
SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC  
(SEQ ID NO 191)  
SUMM \*\*\*MHP\*\*\* -ICG-1: CATGTTGGGCTTGATCGGGTGC  
(SEQ ID NO 191)  
SUMM The invention also provides for a method as described above to detect  
and identify one or more \*\*\*Mycoplasma\*\*\* strains in a sample,  
wherein step (iii) comprises hybridizing to at least one of the  
following probes:  
SUMM \*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC  
(SEQ ID NO 52)  
SUMM and/or to any probe derived from SEQ ID NO 124 or 125 provided said  
probe hybridizes specifically with \*\*\*Mycoplasma\*\*\* species.  
SUMM More particularly, the invention provides for a method as described  
above to detect and identify one or more \*\*\*Mycoplasma\*\*\* pneumoniae  
strains in a sample, wherein step (iii) comprises hybridizing to at  
least one of the following probes:  
SUMM . . . equivalents of said probes, and/or to any probe derived from  
SEQ ID NO 125 provided said probe hybridizes specifically to  
\*\*\*Mycoplasma\*\*\* pneumoniae. According to a preferred embodiment, both  
these probes are used in combination.  
SUMM In another particular embodiment, the invention provides for a method as  
described above to detect and identify one or more \*\*\*Mycoplasma\*\*\*  
genitalium strains in a sample, wherein step (iii) comprises hybridizing  
to the following probe:  
SUMM . . . equivalents of said probes, and/or to any probe derived from  
SEQ ID NO 124 provided said probe hybridizes specifically to  
\*\*\*Mycoplasma\*\*\* genitalium.  
DRWD FIG. 49: represents the DNA sequence of the 16S-23S spacer region from  
\*\*\*Mycoplasma\*\*\* genitalium (U. Gobel) (SEQ ID NO 124)  
DRWD FIG. 50: represents the DNA sequence of the 16S-23S spacer region from  
\*\*\*Mycoplasma\*\*\* pneumoniae ATCC 29432 (SEQ ID NO 125)  
DETD . . . TCGGGCTTGGCCAGAGCTGTT 184  
MKA-ICG-8 GGGTGC GCAACAGCAAGCGA 185  
MKA-ICG-9 GATGCGTTGCCCCTACGGG 186  
MKA-ICG-10 CCCTACGGGTAGCGTGTCTTTTG 187  
MML-ICG-1 CGGATCGATTGAGTGCTTGTCCT 188  
MML-ICG-2 TCTAAATGAACGCACTGCCGATGG 189

MCE-ICG-1 TGAGGGAGCCCGTGCCTGTA 190  
 \*\*\*MHP\*\*\* -ICG-1 CATGTTGGGCTTGATCGGGTGC 191  
 PA-ICG 1 TGGTGTGCTGCGTGATCCGAT 34  
 PA-ICG 2 TGAATGTTTCGTGGATGAACATTGATT 35  
 PA-ICG 3 CACTGGTGATCATTCAAGTCAAG 36  
 PA-ICG 4 TGAATGTTTCGT(G/A)(G/A)ATGAACATTGATTCTGGTC 37  
 PA-ICG 5. . . CHTR-ICG 3 GAGTAGCGTGGTGAGGACGAGA 47  
 CHPS-ICG 1 GGATAACTGTCTTAGGACGGTTTGAC 48  
 MPN-ICG 1 ATCGGTGGTAAATTAACCCAAATCCCTGT 49  
 MPN-ICG 2 CAGTTCTGAAAGAACATTTCCGCTTCTTTC 50  
 MGE-ICG 1 CACCCATTAATTTTTTCGGTGTTAAACCC 51  
 \*\*\*Mycoplasma\*\*\* -ICG CAAAACTGAAAACGACAATCTTTCTAGTTCC 52  
 STAU-ICG 1 TACCAAGCAAAACCGAGTGAATAAAGAGTT 53  
 STAU-ICG 2 CAGAAGATGCGGAATAACGTGAC 54  
 STAU-ICG 3 AACGAAGCCGTATGTGAGCATTTGAC 55  
 STAU-ICG 4 GAACGTAACCTCATGTTAACGTTTGACTTAT 56  
 ACI-ICG 1. . .

DETD . . . *Lactococcus casei* 1 - -

*Escherichia coli* 1 - -

*Hafnia halvei* 1 - -

*Agrobacterium tumefaciens* 2 - -

\*\*\*Mycoplasma\*\*\* *dimorpha* 1 - -

*Clostridium tyrobutyricum* 1 - -

*Clostridium perfringens* 1 - -

*Clostridium sporogenes* 1 - -

*Clostridium*. . .

DETD \*\*\*Mycoplasma\*\*\* *pneumoniae* and \*\*\*Mycoplasma\*\*\* *genitalium*

DETD \*\*\*Mycoplasmas\*\*\* are a group of the smallest prokaryotes known that are able to grow in cell-free media, lack a cell wall,. . .

DETD In humans, \*\*\*mycoplasmas\*\*\* have been recognized either as pathogenic organisms or as commensals. The best known pathogen is \*\*\*Mycoplasma\*\*\* *pneumoniae*, the causative agent of primary atypical pneumonia, especially in children and young adults. The diagnosis of *M. pneumoniae* has. . .

DETD Another pathogen, first isolated from urethral specimens from patients with nongonococcal urethritis, has been described as \*\*\*Mycoplasma\*\*\* *genitalium*. This \*\*\*mycoplasma\*\*\* has several properties in common with *M. pneumoniae*. Both species are pathogenic, and both possess the capability to adhere to. . .

DETD . . . of both species is time-consuming and serology lacks specificity more rapid and more specific assays were developed to identify these \*\*\*mycoplasmas\*\*\*. The use of hybridization assays with DNA probes was described for these species, but despite good specificities these tests do. . .

DETD . . . species deposited in the EMBL databank (MPMAC and MGG37 respectively). Based on this information four probes were derived: one general \*\*\*Mycoplasma\*\*\* probe, two *M. pneumoniae* specific, and one *M. genitalium* specific probe:

DETD \*\*\*Mycoplasma\*\*\* -ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC

DETD . . . SSC, 20% formamide at 50.degree. C.) to amplified spacer-material from four *M. pneumoniae* strains, one *M. genitalium* strain and twenty-two non- \*\*\*Mycoplasma\*\*\* species strains. The general probe hybridized only to the five \*\*\*Mycoplasma\*\*\* strains tested, while the specific probes hybridized only to strains of the species for which they were designed.





China

SO Zhonghua Pifuke Zazhi, (June, 2000) Vol. 33, No. 3, pp. 169-170. print.

ISSN: 0412-4030.

DT Article

LA Chinese

SL Chinese; English

AB Objective: To study the role of *gyrA* gene in quinolone resistant mutants of \*\*\*Mycoplasma\*\*\* hominis (Mh). Methods: Three cross-resistant mutants of \*\*\*Mycoplasma\*\*\* hominis (Mh) to 9 quinolones were selected from 100 clinical strains of Mh by broth dilution method. Their *gyrA* were amplified by PCR, and the nucleotide sequences were compared to those of susceptible strain \*\*\*MhPG21\*\*\* by DNA sequencing. Results: A 350 bp DNA fragment was obtained by PCR. Three resistant mutants contained nucleotide 113 CfwdarwT, 168 TfwdarwA, 183 CfwdarwT, 261 TfwdarwC transitions. Only 113 CfwdarwT transition lead to substitution of Ser-83 by Leu in the *gyrA* protein, the others did not lead to the substitution of amino acid. Conclusion: These results suggest that a *gyrA* mutation in Mh at Ser-83 is associated with quinolone resistance.

TI Study on *gyrA* gene mutations in quinolone resistant \*\*\*Mycoplasma\*\*\* hominis.

AB Objective: To study the role of *gyrA* gene in quinolone resistant mutants of \*\*\*Mycoplasma\*\*\* hominis (Mh). Methods: Three cross-resistant mutants of \*\*\*Mycoplasma\*\*\* hominis (Mh) to 9 quinolones were selected from 100 clinical strains of Mh by broth dilution method. Their *gyrA* were amplified by PCR, and the nucleotide sequences were compared to those of susceptible strain \*\*\*MhPG21\*\*\* by DNA sequencing. Results: A 350 bp DNA fragment was obtained by PCR. Three resistant mutants contained nucleotide 113 CfwdarwT, . . .

BC \*\*\*Mycoplasmataceae\*\*\* 07512

IT Major Concepts

Molecular Genetics (Biochemistry and Molecular Biophysics); Infection

IT Chemicals & Biochemicals

quinolone: resistance; \*\*\*Mycoplasma\*\*\* hominis *gyrA* gene (\*\*\*Mycoplasmataceae\*\*\*)

ORGN Super Taxa

\*\*\*Mycoplasmataceae\*\*\* : \*\*\*Mycoplasmatales\*\*\* ,  
\*\*\*Mycoplasmas\*\*\* , Eubacteria, Bacteria, Microorganisms

ORGN Organism Name

\*\*\*Mycoplasma\*\*\* hominis ( \*\*\*Mycoplasmataceae\*\*\* ): pathogen

ORGN Organism Superterms

Bacteria; Eubacteria; Microorganisms

L10 ANSWER 11 OF 26 MEDLINE

AN 1999209986 MEDLINE

DN 99209986 PubMed ID: 10195611

TI Effect of vaccination against \*\*\*Mycoplasma\*\*\* hyopneumoniae in pig herds with an all-in/all-out production system.

AU Maes D; Deluyker H; Verdonck M; Castryck F; Miry C; Vrijens B; Verbeke W; Viaene J; de Kruif A

CS Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, University of Ghent, Merelbeke, Belgium.

SO VACCINE, (1999 Mar 5) 17 (9-10) 1024-34.

Journal code: 8406899. ISSN: 0264-410X.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English  
FS Priority Journals  
EM 199906

ED Entered STN: 19990614  
Last Updated on STN: 19990614  
Entered Medline: 19990602

AB A multi-site field study was conducted to evaluate an inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae (Mh) vaccine in 14 pig herds infected by Mh and practising an all-in/all-out production system. In each herd, a vaccinated and control group of 250 pigs each were compared during the growing/finishing period with respect to performance parameters (major variables) and by means of clinical, serological and pathological parameters (ancillary variables). Mh vaccination significantly ( $P < 0.05$ ) improved daily weight gain (+22 g), feed conversion ratio (-0.07), medication costs (-0.476 ECU/pig) (1 ECU = US\$1.0269542), prevalence of pneumonia lesions (-14%) and severity of pneumonia lesions (-3%). Mortality rate, severity of coughing and carcass quality were not significantly influenced by Mh vaccination. Serological results of Mh and other respiratory pathogens are presented and discussed. A cost-benefit analysis based on significantly improved performance parameters demonstrated that Mh vaccination was economically attractive as it resulted in an increase of the net return to labour with 1.300 ECU per finishing pig sold. The sensitivity of the economic benefit was illustrated towards fluctuations in pig finishing prices.

TI Effect of vaccination against \*\*\*Mycoplasma\*\*\* hyopneumoniae in pig herds with an all-in/all-out production system.

AB A multi-site field study was conducted to evaluate an inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae (Mh) vaccine in 14 pig herds infected by Mh and practising an all-in/all-out production system. In each herd, a .

CT . . . Female; Support, Non-U.S. Gov't  
Antibodies, Bacterial: BI, biosynthesis  
Bacterial Proteins: IM, immunology  
\*Bacterial Vaccines  
Bacterial Vaccines: EC, economics  
Cost-Benefit Analysis  
\*\*\*\*Mycoplasma: IM, immunology\*\*\*  
\*\*\* Pneumonia, Mycoplasma: PC, prevention & control\*\*\*  
\*\*\*\*Pneumonia, Mycoplasma: VE, veterinary\*\*\*  
Swine  
\*Swine Diseases: PC, prevention & control  
\*Vaccination  
Vaccination: EC, economics  
Weight Gain

CN 0 (Antibodies, Bacterial); 0 (Bacterial Proteins); 0 (Bacterial Vaccines);  
0 ( \*\*\*Mhp1\*\*\* protein, \*\*\*Mycoplasma\*\*\* )

L10 ANSWER 12 OF 26 CABA COPYRIGHT 2002 CABI

AN 2000:51240 CABA

DN 20002209387

TI Comparison of protein profiles and chromosomal DNA of \*\*\*Mycoplasma\*\*\* hyopneumoniae strains by SDS-PAGE and PFGE

AU Shao GuoQing; Qian JianFei; Lin Wen; Yang Kui; Zhao Jun; Cai BaoXiang;  
Shao, G. Q.; Qian, J. F.; Lin, W.; Yang, K.; Zhao, J.; Cai, B. X.

CS Institute of Animal Husbandry and Veterinary Science, Jiangsu Academy of

Agricultural Sciences, Nanjing 210014, China.  
 SO Jiangsu Journal of Agricultural Sciences, (1999) Vol. 15, No. 4, pp.  
 247-249. 10 ref.  
 ISSN: 1000-4440  
 DT Journal  
 LA Chinese  
 SL English  
 AB is the primary agent of \*\*\*Mycoplasma\*\*\* (or enzootic) pneumonia of swine (MPS) and one of the most important direct or indirect causes of porcine respiratory disease. Three \*\*\*Mycoplasma\*\*\* strains, \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ) 168, an artificially attenuated strain, a field strain, isolated and identified from a pig with MPS and proved to be virulent and a model strain J were grown in KM2. After 48-72 h incubation at 37 deg C, cells were harvested through centrifugation, then washed and suspended in 0.01 mol/litre PBS to 1/100 of the original culture volume. Suspended \*\*\*Mycoplasma\*\*\* solutions solubilized with sodium dodecyl sulfate (SDS), and protein profiles of the \*\*\*Mycoplasmas\*\*\* were showed using SDS-PAGE. The three \*\*\*Mhp\*\*\* strains from diverse origin produced similar protein profiles, suggesting that virulent and avirulent \*\*\*Mhp\*\*\* strains might possess a species-specific protein profile. Full-size chromosomal DNA of Mph strains 168 and J were prepared by LMP agarose-embedment lysis cell membrane method, chromosomal DNA size were estimated as 1000 kb by pulsed field gel electrophoresis (PFGE) from the mobilities of these full-length molecules compared with DNA size references, Saccharomyces cerevisiae, PFGE results indicated that either \*\*\*Mhp\*\*\* strain or strain J had only one chromosomal DNA band.  
 TI Comparison of protein profiles and chromosomal DNA of \*\*\*Mycoplasma\*\*\* hyopneumoniae strains by SDS-PAGE and PFGE.  
 AB is the primary agent of \*\*\*Mycoplasma\*\*\* (or enzootic) pneumonia of swine (MPS) and one of the most important direct or indirect causes of porcine respiratory disease. Three \*\*\*Mycoplasma\*\*\* strains, \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ) 168, an artificially attenuated strain, a field strain, isolated and identified from a pig with MPS and proved to be. . . were harvested through centrifugation, then washed and suspended in 0.01 mol/litre PBS to 1/100 of the original culture volume. Suspended \*\*\*Mycoplasma\*\*\* solutions solubilized with sodium dodecyl sulfate (SDS), and protein profiles of the \*\*\*Mycoplasmas\*\*\* were showed using SDS-PAGE. The three \*\*\*Mhp\*\*\* strains from diverse origin produced similar protein profiles, suggesting that virulent and avirulent \*\*\*Mhp\*\*\* strains might possess a species-specific protein profile. Full-size chromosomal DNA of Mph strains 168 and J were prepared by LMP. . . (PFGE) from the mobilities of these full-length molecules compared with DNA size references, Saccharomyces cerevisiae, PFGE results indicated that either \*\*\*Mhp\*\*\* strain or strain J had only one chromosomal DNA band.  
 BT \*\*\*Mycoplasmataceae\*\*\* ; \*\*\*Mycoplasmatales\*\*\* ; Mollicutes; Tenericutes; bacteria; prokaryotes; \*\*\*Mycoplasma\*\*\* ; Sus scrofa; Sus; Suidae; Suiformes; Artiodactyla; mammals; vertebrates; Chordata; animals; ungulates; Endomycetales; Ascomycotina; Eumycota; fungi; Saccharomyces; East Asia; Asia; Developing. . .  
 ORGN \*\*\*Mycoplasma\*\*\* ; \*\*\*Mycoplasma\*\*\* hyopneumoniae; pigs; Saccharomyces; Saccharomyces cerevisiae

AN 1998:75990 CAPLUS

DN 128:150361

TI PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae

IN Artiushin, Sergey; Stipkovits, Laszlo; Minion, F. Chris

PA Iowa State University Research Foundation, Inc., USA

SO U.S., 12 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI US 5712090	A	19980127	US 1993-62632	19930518
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AB A PCR-based assay is disclosed for \*\*\*Mycoplasma\*\*\* hyopneumoniae using a species-specific primer pair and a related diagnostic kit. The primer pair is made up of an oligonucleotide having the nucleotide sequence 5'-AAGTTCATTCGCGCTAGCCC-3' and an oligonucleotide having the nucleotide sequence 5'-GCTCCTACTCCATATTGCCC-3'. Preferably, the kit contains an oligonucleotide probe having the sequence 5'-GGTAGCCCTTCCTTTGAGGT-3'. Based upon DNA sequence anal. of a cloned fragment of \*\*\*Mhp\*\*\* DNA, the PCR primers were constructed and tested against different strains of \*\*\*Mhp\*\*\*, \*\*\*Mycoplasma\*\*\* flocculare, other \*\*\*mycoplasma\*\*\* species, and non-Mollicute microorganisms which commonly inhabit the respiratory tracts of swine. A total of 40 field isolates from \*\*\*Mhp\*\*\* and 4 field isolates of M. flocculare were examd. Pos. signals were obtained in PCR with \*\*\*Mhp\*\*\* ref. strains and all 40 \*\*\*Mhp\*\*\* field isolates, but not with other Mollicutes or microorganisms.

TI PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae

AB A PCR-based assay is disclosed for \*\*\*Mycoplasma\*\*\* hyopneumoniae using a species-specific primer pair and a related diagnostic kit. The primer pair is made up of an oligonucleotide having the nucleotide sequence 5'-AAGTTCATTCGCGCTAGCCC-3' and an oligonucleotide having the nucleotide sequence 5'-GCTCCTACTCCATATTGCCC-3'. Preferably, the kit contains an oligonucleotide probe having the sequence 5'-GGTAGCCCTTCCTTTGAGGT-3'. Based upon DNA sequence anal. of a cloned fragment of \*\*\*Mhp\*\*\* DNA, the PCR primers were constructed and tested against different strains of \*\*\*Mhp\*\*\*, \*\*\*Mycoplasma\*\*\* flocculare, other \*\*\*mycoplasma\*\*\* species, and non-Mollicute microorganisms which commonly inhabit the respiratory tracts of swine. A total of 40 field isolates from \*\*\*Mhp\*\*\* and 4 field isolates of M. flocculare were examd. Pos. signals were obtained in PCR with \*\*\*Mhp\*\*\* ref. strains and all 40 \*\*\*Mhp\*\*\* field isolates, but not with other Mollicutes or microorganisms.

ST PCR detection \*\*\*Mycoplasma\*\*\* hyopneumoniae

IT \*\*\*Mycoplasma\*\*\* hyopneumoniae

PCR (polymerase chain reaction)

(PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae)

IT Primers (nucleic acid)

Probes (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae)

IT Nucleic acid hybridization

(of amplified DNA in PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae)

IT 154339-48-7 154339-49-8

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(primer; PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae)

IT 202539-35-3

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(probe; PCR-based assay for \*\*\*Mycoplasma\*\*\* hyopneumoniae)

L10 ANSWER 14 OF 26 USPATFULL

AN 1998:112145 USPATFULL

TI Orally administrable formulations of quinolone- or  
naphthyridonecarboxylic acids

IN Vetter, Oliver, Burscheid, Germany, Federal Republic of  
Lohr, Reinhold, Bergisch Gladbach, Germany, Federal Republic of  
Kuhn, Matthias, Monheim, Germany, Federal Republic of  
Rast, Hubert, Leverkusen, Germany, Federal Republic of  
Scheer, Martin, Wuppertal, Germany, Federal Republic of  
Heinen, Ernst, Echternacherbruuck, Germany, Federal Republic of  
Cabrera, Francisco, Overland Park, KS, United States

PA Bayer Aktiengesellschaft, Leverkusen, Germany, Federal Republic of  
(non-U.S. corporation)

PI US 5808076 19980915

AI US 1996-761687 19961206 (8)

PRAI DE 1996-19633480 19960820

DT Utility

FS Granted

EXNAM Primary Examiner: Dentz, Bernard; Assistant Examiner: Aulakh, Charanjit  
S.

LREP Sprung Kramer Schaefer & Briscoe

CLMN Number of Claims: 9

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 420

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to the preparation of orally administrable  
formulations of quinolone- or naphthyridonecarboxylic acids by mixing  
quinolone- or naphthyridonecarboxylic acids as such or in the form of  
their water-soluble salts or derivatives, preferably in the form of  
their aqueous salt solutions, with embonic acid as such or in the form  
of its water-soluble salts or derivatives, preferably in the form of its  
aqueous salt solutions, in the presence of an excipient, optionally in  
the presence of auxiliaries, when using dry mixtures in the presence of  
water, and optionally converting the mixture obtained into further  
ready-to-use forms.

SUMM 18. \*\*\*Mycoplasmataceae\*\*\* (for example \*\*\*Mycoplasma\*\*\*  
mycoides, M. agalactiae, M. gallisepticum)

SUMM . . . pyogenes; tuberculosis in cattle, swine, fowl and other kinds  
of animals; paratuberculosis of the ruminants; nocardiosis; Q fever;  
ornithosis-psittacosis; encephalomyelitis; \*\*\*mycoplasmosis\*\*\* of  
cattle and other animals, porcine enzootic pneumonia.

DETD . . . -- 0.670

sulfonic acid

sodium sulfite

0.10 0.10 0.025 0.025

embonic acid

10.82 9.74 2.705 4.057

methyl-	10.88	--	2.720	4.080
glucamine				
arginine	--	8.75	--	--
***MHP***	-	--	--	1.500 1.500
cellulose	50			
Ac-Di-Sol				
	7.00	7.00	--	--
corn starch				
	100.0	100.0	100.0	100.0
moist ad				
(weight in g)				

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L10 ANSWER 15 OF 26 USPATFULL

AN 1998:91598 USPATFULL

TI DNA sequences coding for \*\*\*mycoplasma\*\*\* hyopneumoniae surface antigens, corresponding proteins and use in vaccines and diagnostic procedures

IN Wise, Kim S., Columbia, MO, United States

McIntosh, Mark A., Columbia, MO, United States

PA The Curators of the University of Missouri, Columbia, MO, United States (U.S. corporation)

PI US 5788962 19980804

AI US 1996-703947 19960828 (8)

RLI Continuation of Ser. No. US 1995-373957, filed on 17 Jan 1995, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer

LREP Fishel, Grace J.

CLMN Number of Claims: 3

ECL Exemplary Claim: 1

DRWN 9 Drawing Figure(s); 17 Drawing Page(s)

LN.CNT 713

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 surface antigens prepared by recombinant DNA or synthetic methods, protein antigens encoded by P65 gene, an expression vector and transformed host containing the antigens, a vaccine based on such antigens, methods of treating swine, etc. to prevent enzootic pneumonia using that vaccine and diagnostic tests to detect the presence of \*\*\*Mycoplasma\*\*\* hyopneumoniae.

TI DNA sequences coding for \*\*\*mycoplasma\*\*\* hyopneumoniae surface antigens, corresponding proteins and use in vaccines and diagnostic procedures

AB \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 surface antigens prepared by recombinant DNA or synthetic methods, protein antigens encoded by P65 gene, an expression vector. . . methods of treating swine, etc. to prevent enzootic pneumonia using that vaccine and diagnostic tests to detect the presence of \*\*\*Mycoplasma\*\*\* hyopneumoniae.

SUMM The present invention relates to \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 surface antigen, a lipoprotein capable of eliciting an antibody or other immune response which selectively recognizes an epitope(s) of P65, to such antigens prepared by recombinant DNA methods, to swine \*\*\*mycoplasma\*\*\* vaccine based on such antigens, to methods of treating swine to prevent enzootic pneumonia using that vaccine, and to

diagnostic tests for detecting the presence of swine \*\*\*mycoplasma\*\*\* infections in swine herds.

SUMM Enzootic pneumonia in swine is caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae. The disease is transmitted from pig to pig through the nasal passages by airborne organisms expelled from the lungs of infected pigs. The primary infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae may be followed by secondary infection by other \*\*\*mycoplasma\*\*\* species (\*\*\*Mycoplasma\*\*\* hyorhinis and \*\*\*Mycoplasma\*\*\* flocculare) as well as bacterial pathogens. The disease rarely causes death but causes decreased growth and weight gain.

SUMM \*\*\*Mycoplasma\*\*\* hyopneumoniae is a small, prokaryotic microbe smaller and simpler in structure than bacteria, but more complex than viruses. Unlike viruses, . . . eukaryotic cells as they have absolute requirements for exogenous sterols and fatty acids which generally necessitates growth in serum-containing media. \*\*\*Mycoplasma\*\*\* hyopneumoniae is bounded by a cell membrane but not by a cell wall. They have an extremely small genome, approximately. . .

SUMM Due to the serious economic consequences of pig pneumonia, vaccines against \*\*\*Mycoplasma\*\*\* hyopneumoniae and diagnostic testing methods which will indicate the presence of an infection have been sought. Vaccines containing preparations of \*\*\*mycoplasma\*\*\* organisms grown in broth medium have been marketed. However, the requirement for serum in growth medium adds great expense to. . . concerns regarding adverse reactions induced by serum components present in the immunizing material. Cost effective production of the appropriate recombinant \*\*\*mycoplasma\*\*\* protein in the absence of serum offers an improvement in terms of economy and vaccine safety. Such a recombinant protein. . . agent. Current serologic assays for detection are confounded by non-specific reactions due to the antigenic cross-reactivity of multiple common swine \*\*\*mycoplasmas\*\*\*.

SUMM In earlier work, certain lipoproteins present on the cellular membrane of \*\*\*Mycoplasma\*\*\* hyopneumoniae were identified as mediating or inducing immunological reactions (Kim, M. F., M. B. Heidari, S. J. Stull, M. A.. . . be capable of eliciting an antibody which selectively recognized an epitope(s) of P65 without unwanted cross-reactions with antibodies to other \*\*\*mycoplasmas\*\*\*, especially \*\*\*Mycoplasma\*\*\* flocculare. P65 was shown to be integrally associated with the cell membrane and covalently linked to a lipid moiety, with. . .

SUMM . . . an attractive candidate for developing a recombinant protein because of its ability to elicit an antibody which is selective for \*\*\*Mycoplasma\*\*\* hyopneumoniae, attempts to determine the amino acid sequences failed because of N-terminal blocking by the lipid and isolation of internal. . .

SUMM Determining and utilizing the DNA sequence coding for P65 has also been very difficult, despite the small size of the \*\*\*mycoplasma\*\*\* chromosome, because of a lack of vehicles for mobilizing and transferring DNA into these organisms (Kim et al, 1990, supra) and because of inherent problems in the expression of \*\*\*mycoplasma\*\*\* genes in more versatile, heterologous hosts such as E. coli. \*\*\*Mycoplasma\*\*\* hyopneumoniae uses an alternative genetic code (in which the UGA translation termination codon is used to encode tryptophan) and expression of \*\*\*mycoplasma\*\*\* genes in E. coli is complicated by truncation of products at the UGA codons. In addition, expression of partial products occurs through promiscuous translation

from multiple, unpredictable sites in structural genes, a phenomenon that is probably common among most \*\*\*mycoplasmas\*\*\* (Notarnicola, S. N., M. A. McIntosh, and K. S. Wise. 1990. J. Bacteriol. 172:2986-2995).

SUMM . . . sequences described above; a diagnostic procedure based on those proteins or antibodies raised against them for detecting the presence of \*\*\*Mycoplasma\*\*\* hyopneumoniae infections in swine herds, and a diagnostic procedure utilizing any portion of said DNA sequence shown in FIG. 1. . .

DRWD . . . depicts the DNA sequence encoding the entire 627 amino acids of the structural gene for the surface lipoprotein P65 from \*\*\*Mycoplasma\*\*\* hyopneumoniae and includes 312 base pairs upstream and 479 base pairs downstream of the coding sequence. The DNA sequence in. . .

DRWD FIG. 5 is a schematic showing the structure and features of \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 lipoprotein gene.

DRWD . . . of P65 by the Mab defining this protein, and the selective recognition of the recombinant forms by swine infected with \*\*\*Mycoplasma\*\*\* hyopneumoniae. The three panels are Western blots of identical samples, immunostained with the Mab defining P65 (left panel), serum from swine after experimental inoculation with \*\*\*Mycoplasma\*\*\* hyopneumoniae (central panel), or serum from the same swine prior to inoculation (right panel). Antibodies and sera are described in. . . factor Xa to generate the rP65 fragment (arrows) (middle lane of each panel), or Triton X-114 phase membrane proteins of \*\*\*Mycoplasma\*\*\* hyopneumoniae (right lane of each panel). Minor bands in the left panel are breakdown products of the predominant FP65 or. . .

DETD . . . and 2193 as shown in FIG. 1, (SEQ ID NO:1) the complete 2672 sequence illustrating the entire structural gene for \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 including its hydrophobic lipid-modified N-terminal end. More generally, the invention concerns any DNA sequence coding for a protein. . .

DETD . . . the art and used in a vaccine for protection of a non-human animal, such as a bovine, swine, etc., against \*\*\*mycoplasma\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae. Said one or more proteins which are capable of eliciting an antibody or other immune response which recognizes an. . . amino acid sequence depicted in FIG. 2 (SEQ ID NO:2) are used in an amount effective to provide protection against \*\*\*mycoplasma\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae and may be used in combination with a suitable physiologically acceptable carrier.

DETD The term "protecting" or "protection" when used with respect to the vaccine for \*\*\*mycoplasma\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae described herein means that the vaccine prevents \*\*\*mycoplasma\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae or reduces the severity of the disease.

DETD The present invention provides a method of immunizing a susceptible non-human animal, e.g., swine, bovine, etc., against \*\*\*mycoplasma\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae with the vaccine described above. For purposes of this invention, the vaccine is administered in an effective amount. The. . .

DETD . . . has been vaccinated with the vaccine of the present invention, other vaccines containing P65 protein, or is infected with naturally-occurring \*\*\*Mycoplasma\*\*\* hyopneumoniae. This method comprises obtaining from the animal a sample of suitable body fluid or



tissue, detecting in the sample a presence of antibodies or other immune responses to \*\*\*Mycoplasma\*\*\* hyopneumoniae by reaction with a protein having an amino acid sequence depicted in FIG. 2 (SEQ ID NO:2), a fragment. . .

DETD Alternatively, the fluid sample may be tested for the presence of a gene for \*\*\*Mycoplasma\*\*\* hyopneumoniae P65 by reaction with a recombinant or synthetic DNA sequence contained within the sequence shown in FIG. 1 (SEQ. . .

DETD Identification, Characterization and Modification of the \*\*\*Mycoplasma\*\*\* Hyopneumoniae P65 Structural Gene

DETD . . . to nt2193) which contained three internal, in-frame UGA codons (beginning at nt634, nt865, and nt1237) that designate tryptophan incorporation in \*\*\*mycoplasma\*\*\* proteins. These codons would prevent expression of the full-length P65 protein in E. coli. Downstream of these codons, positions for. . . a calculated molecular weight of 71,026, with a 29-amino acid hydrophobic signal sequence responsible for translocating the protein to the \*\*\*mycoplasma\*\*\* cell surface. The signal domain is bounded on its downstream end by the amino acid sequence SAGC which represents a. . .

DETD . . . was shown to produce a P65 polypeptide in E. coli that contained the entire amino acid sequence of the mature \*\*\*mycoplasma\*\*\* protein and could be detected by polyclonal and monoclonal antibody (MAb) to P65.

DETD To ensure a soluble product, yet to include all external, hydrophilic sequences of the authentic \*\*\*mycoplasma\*\*\* P65 protein, a selected region of the P65 gene was used for this fusion in which the signal peptide sequence. . .

DETD Demonstration of the Appropriate Recombinant Products, Recognition of Products by Antibodies of Swine Infected with \*\*\*Mycoplasma\*\*\* Hyopneumoniae and Solubility Properties

DETD . . . the fusion protein, at approximately 43 kDa; data not shown). The liberated rP65 product was slightly larger than the authentic \*\*\*mycoplasma\*\*\* P65, as predicted from additional residues encoded by vector sequences that are absent from the processed form of the mature protein in \*\*\*mycoplasma\*\*\*. This is shown by staining the rP65 product and detergent phase proteins from the \*\*\*mycoplasma\*\*\* (hpn TX) with Mab to P65 (compare the middle and right lanes of the left panel in FIG. 8). In. . .

DETD The solubility properties of both FP65 and rP65 are shown in comparison to the authentic, lipid modified \*\*\*mycoplasma\*\*\* version, using detergent fractionation (Wise and Kim, 1987, supra and Kim et al 1990, supra). FIG. 9 shows that the. . .

DETD . . . genomic DNA

(A) DESCRIPTION: region of 5.8 kb HindIII fragment from genomic library

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: \*\*\*Mycoplasma\*\*\* hyopneumoniae

(B) STRAIN: J

(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE: unicellular bacterium  
 (H) CELL LINE:  
 (I) ORGANELLE:  
 (vii) IMMEDIATE SOURCE:  
 (A) LIBRARY: Genomic in .gamma. Charon 4A, .gamma. GEM12  
 (B) CLONE: .gamma. \*\*\*MhpJ25\*\*\* , .gamma. \*\*\*MhpJ35\*\*\* , .gamma.  
 \*\*\*MhpJG35\*\*\* ,  
 pZJ25, pZJ25.1, pZ125.14,  
 pZJG35.1, pZJG35.12, pZJG35.13,  
 pZJG35.14  
 (viii) POSITION IN GENOME:  
 (A) CHROMOSOME/SEGMENT: single chromosome  
 (B) MAP POSITION: unknown  
 (C) UNITS: unknown  
 (ix) FEATURE:  
 (A) NAME/KEY: sequence encodes entire. . . Kim, Manijeh B. Heidari,  
 Susan J. Stull, Mark A.  
 McIntosh, and Kim S. Wise  
 (B) TITLE: Identification and Mapping of an  
 Immunogenic Region of \*\*\*Mycoplasma\*\*\*  
 hyopneumoniae p65 Surface  
 Lipoprotein Expressed in  
 Escherichia coli from a Cloned  
 Genomic Fragment  
 (C) JOURNAL: Infection and Immunity  
 (D) VOLUME: 58  
 (E) ISSUE: 8  
 (F) PAGES: 2637- 2643  
 (G). . .  
 DETD . . . residues of the P65  
 lipoprotein, derived from the  
 nucleic acid sequence  
 (iii) HYPOTHETICAL: no  
 (iv) ANTI-SENSE: no  
 (v) FRAGMENT TYPE: whole polypeptide  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: \*\*\*Mycoplasma\*\*\* hyopneumoniae  
 (B) STRAIN: J  
 (C) INDIVIDUAL ISOLATE:  
 (D) DEVELOPMENTAL STAGE:  
 (E) HAPLOTYPE:  
 (F) TISSUE TYPE:  
 (G) CELL TYPE: unicellular bacterium  
 (H) CELL LINE:  
 (I) ORGANELLE:  
 (vii) IMMEDIATE SOURCE:  
 (A) LIBRARY: Genomic in .gamma. Charon 4A, .gamma. GEM12  
 (B) CLONE: .gamma. \*\*\*MhpJ25\*\*\* , .gamma. \*\*\*MhpJ35\*\*\* , .gamma.  
 \*\*\*MhpJG35\*\*\* ,  
 pZJ25, pZJ25.1, pZJ25.14,  
 pZJG35.1, pZJG35.12, pZJG35.13,  
 pZJG35.14  
 (viii) POSITION IN GENOME:  
 (A) CHROMOSOME/SEGMENT: single chromosome  
 (B) MAP POSITION: unknown

(C) UNITS: unknown

(ix) FEATURE:

(A) NAME/KEY: 627 amino acid. . . Kim, Manijeh B. Heidari,  
Susan J. Stull, Mark A.

McIntosh, and Kim S. Wise

(B) TITLE: Identification and Mapping of an  
Immunogenic Region of \*\*\*Mycoplasma\*\*\*  
hyopneumoniae p65 Surface  
Lipoprotein Expressed in  
Escherichia coli from a Cloned  
Genomic Fragment

(C) JOURNAL: Infection and Immunity

(D) VOLUME: 58

(E) ISSUE: 8

(F) PAGES: 2637- 2643

(G). . .

CLM What is claimed is:

1. A vaccine for protecting a susceptible swine against  
\*\*\*mycoplasmal\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\*  
hyopneumoniae comprising an immunogenic fusion protein in a suitable  
physiologically acceptable carrier, said fusion protein having a first  
amino acid. . .
2. A method for inducing an immune response in a susceptible swine  
against \*\*\*mycoplasmal\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\*  
hyopneumoniae, comprising administering to the swine a composition  
comprising an isolated and purified protein having the amino acid  
sequence depicted. . . (SEQ ID NO:2), immunogenic fragments or  
immunogenic fusion proteins thereof in an amount effective for producing  
an immune response against \*\*\*mycoplasmal\*\*\* pneumonia caused by  
\*\*\*Mycoplasma\*\*\* hyopneumoniae.
3. A vaccine for protecting a susceptible swine against  
\*\*\*mycoplasmal\*\*\* pneumonia caused by \*\*\*Mycoplasma\*\*\*  
hyopneumoniae comprising an immunogenic fusion protein in a suitable  
physiologically acceptable carrier, said fusion protein having a first  
amino acid. . .

L10 ANSWER 16 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

3

AN 1998:391142 BIOSIS

DN PREV199800391142

TI Reiterated repeat region variability in the ciliary adhesin gene of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae.

AU Wilton, Jody L.; Scarman, Anthony L.; Walker, Mark J.; Djordjevic, Steven  
P. (1)

CS (1) Microbiol. and Immunol. Sect., Elizabeth Macarthur Agric. Inst., PMB  
8, Camden, NSW 2570 Australia

SO Microbiology (Reading), (July, 1998) Vol. 144, No. 7, pp. 1931-1943.  
ISSN: 1350-0872.

DT Article

LA English

AB \*\*\*Mycoplasma\*\*\* hyopneumoniae is a highly prevalent pathogen which  
colonizes the ciliated epithelial lining of the porcine respiratory tract.  
Expression libraries constructed from genomic DNA of the non-pathogenic  
strain M. hyopneumoniae J were screened with porcine hyperimmune antiserum

against *M. hyopneumoniae*. One clone expressed a 28 kDa protein which was also reactive with monospecific antiserum raised against a putative *M. hyopneumoniae*-specific 94 kDa antigen derived from strain J. Trypsin digestion of whole *M. hyopneumoniae* cells showed the 94 kDa antigen to be surface-accessible. DNA sequence analysis of the gene encoding the 94 kDa antigen revealed greater than 90% homology to two adhesin genes, encoding P97 and \*\*\*Mhp1\*\*\*, cloned from pathogenic strain 232 and strain P5722 of *M. hyopneumoniae*, respectively. Two regions of repetitive DNA sequence were identified in the gene encoding the 94 kDa antigen. The first encoded the deduced amino acid sequence A(T)-K-P-E(V)-A(T) arranged as nine tandem repeats (RR1). The second region of repetitive DNA sequence encoded the deduced amino acid sequence G-A(E,S)-P-N(S)-Q-G-K-K-A-E arranged as five tandem repeats (RR2). Comparison of the three *M. hyopneumoniae* adhesin genes revealed that the genes encoding P97 and \*\*\*Mhp1\*\*\*, and the strain J gene encoding the 94 kDa antigen contained 15, 12 and 9 tandem repeats, respectively, in RR1, and 4, 5 and 5 tandem repeats, respectively, in RR2. Southern hybridization analysis of EcoRI-digested genomic DNA probed with an 820 bp fragment spanning RR1 and RR2 identified a strongly hybridizing fragment ranging in size from 2.15 to 2.30 kb among seven geographically diverse strains of *M. hyopneumoniae* but failed to hybridize with DNA from four strains of \*\*\*Mycoplasma\*\*\* hyorhinis or \*\*\*Mycoplasma\*\*\* flocculare strain Ms42. PCR primers flanking the DNA sequence encoding RR1 and RR2 were used to amplify DNA from the seven strains of *M. hyopneumoniae* and DNA sequence analysis of the amplification products showed that the number of tandem amino acid repeats in RR1 varied considerably between strains. RR1 from *M. hyopneumoniae* strains YZ, Beaufort, Sue, OMZ407 and C1735/2 comprised 11, 15, 12, 15 and 8 tandem copies, respectively, of the 5-aa repeat whilst RR2 comprised 4, 3, 4, 3 and 4 tandem copies, respectively, of the 10-aa repeat. Two putative integrin binding sites (L-E-T and R-X-X-X-D) were identified in the 94 kDa ciliary adhesin. Variability in the number of amino acid repeats in RR1 amongst strains of *M. hyopneumoniae* may influence ciliary binding.

TI Reiterated repeat region variability in the ciliary adhesin gene of \*\*\*Mycoplasma\*\*\* *hyopneumoniae*.

AB \*\*\*Mycoplasma\*\*\* *hyopneumoniae* is a highly prevalent pathogen which colonizes the ciliated epithelial lining of the porcine respiratory tract. Expression libraries constructed. . . of the gene encoding the 94 kDa antigen revealed greater than 90% homology to two adhesin genes, encoding P97 and \*\*\*Mhp1\*\*\*, cloned from pathogenic strain 232 and strain P5722 of *M. hyopneumoniae*, respectively. Two regions of repetitive DNA sequence were identified. . . as five tandem repeats (RR2). Comparison of the three *M. hyopneumoniae* adhesin genes revealed that the genes encoding P97 and \*\*\*Mhp1\*\*\*, and the strain J gene encoding the 94 kDa antigen contained 15, 12 and 9 tandem repeats, respectively, in RR1,. . . 2.30 kb among seven geographically diverse strains of *M. hyopneumoniae* but failed to hybridize with DNA from four strains of \*\*\*Mycoplasma\*\*\* hyorhinis or \*\*\*Mycoplasma\*\*\* flocculare strain Ms42. PCR primers flanking the DNA sequence encoding RR1 and RR2 were used to amplify DNA from the. . .

BC \*\*\*Mycoplasmataceae\*\*\* 07512  
Suidae 85740

ORGN Super Taxa

\*\*\*Mycoplasmataceae\*\*\* : \*\*\*Mycoplasmatales\*\*\* ,  
\*\*\*Mycoplasmas\*\*\* , Eubacteria, Bacteria, Microorganisms; Suidae:  
Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name

pig (Suidae); \*\*\*Mycoplasma\*\*\* -hyopneumoniae (  
\*\*\*Mycoplasmataceae\*\*\* ): veterinary pathogen

ORGN Organism Superterms

Animals; Artiodactyls; Bacteria; Chordates; Eubacteria; Mammals;  
Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Vertebrates

L10 ANSWER 17 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

4

AN 1997:118765 BIOSIS

DN PREV199799425268

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's  
vaccine potential.

AU King, Kendall W. (1); Faulds, Daryl H.; Rosey, Everett L.; Yancey, Robert  
J., Jr.

CS (1) 213 Schuring Road, Portage, MI 49024 USA

SO Vaccine, (1997) Vol. 15, No. 1, pp. 25-35.

ISSN: 0264-410X.

DT Article

LA English

AB The gene encoding \*\*\*Mhp1\*\*\* , a 124 kDa protein from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
product characterized. No significant homology to the gene or encoded  
polypeptide was found in the Genbank, NBRF, or PIR databases, though this  
protein appears similar to p97, a putative adhesin of M. hyopneumoniae  
described by Zhang et al. (Infect. Immun. 63, 1013-1019, 1995). Two  
repeated motifs were identified within the 3' end of the gene and encoded  
polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
expression and purification of the protein. Both the authentic and  
recombinant proteins were recognized by sera from pigs infected with M.  
hyopneumoniae. In an induced-disease model in pigs, coughing was reduced  
in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\* , although  
differences were not significant. Only minimal protection against lung  
lesion formation was provided, and again differences between the  
\*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

TI Characterization of the gene encoding \*\*\*Mhp1\*\*\* from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae and examination of \*\*\*Mhp1\*\*\* 's  
vaccine potential.

AB The gene encoding \*\*\*Mhp1\*\*\* , a 124 kDa protein from  
\*\*\*Mycoplasma\*\*\* hyopneumoniae, has been cloned, sequenced, and its  
product characterized. No significant homology to the gene or encoded  
polypeptide was found. . . Immun. 63, 1013-1019, 1995). Two repeated  
motifs were identified within the 3' end of the gene and encoded  
polypeptide. The \*\*\*mhp1\*\*\* gene was fused to the glutathione  
S-transferase (GST) gene from Schistosoma japonicum, enabling high-level  
expression and purification of the protein.. . . from pigs infected  
with M. hyopneumoniae. In an induced-disease model in pigs, coughing was  
reduced in animals vaccinated with recombinant GST- \*\*\*Mhp1\*\*\* ,  
although differences were not significant. Only minimal protection against  
lung lesion formation was provided, and again differences between the  
\*\*\*Mhp1\*\*\* -vaccinated and nonvaccinated groups were not significant.

BC \*\*\*Mycoplasmataceae\*\*\* 07512  
Suidae \*85740

IT Sequence Data  
 amino acid sequence; nucleotide sequence

IT Miscellaneous Descriptors  
 BACTERIAL DISEASE; CHARACTERIZATION; \*\*\*MHP1\*\*\* GENE; \*\*\*MHP1\*\*\*  
 PROTEIN; MOLECULAR GENETICS; \*\*\*MYCOPLASMA\*\*\* HYOPNEUMONIAE  
 INFECTION; PHARMACOLOGY; VACCINE POTENTIAL

ORGN Super Taxa  
 Bacteria - General Unspecified: Eubacteria, Bacteria;  
 \*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria; Suidae: Artiodactyla,  
 Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name  
 bacteria (Bacteria - General Unspecified); pig (Suidae);  
 \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mycoplasmataceae\*\*\* )

ORGN Organism Superterms  
 animals; artiodactyls; bacteria; chordates; eubacteria; mammals;  
 microorganisms; nonhuman mammals; nonhuman vertebrates; vertebrates

L10 ANSWER 18 OF 26 CAPLUS COPYRIGHT 2002 ACS

AN 1996:184244 CAPLUS

DN 124:222848

TI Simultaneous detection, identification and differentiation of eubacterial  
 taxa using a hybridization assay

IN Jannes, Geert; Rossau, Rudi; Van Heuverswyn, Hugo

PA Innogenetics, N.V., Belg.

SO PCT Int. Appl., 247 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9600298	A1	19960104	WO 1995-EP2452	19950623
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2193101	AA	19960104	CA 1995-2193101	19950623
AU 9529246	A1	19960119	AU 1995-29246	19950623
EP 769068	A1	19970423	EP 1995-924923	19950623
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
BR 9508101	A	19971230	BR 1995-8101	19950623
JP 10501976	T2	19980224	JP 1995-502804	19950623
RU 2154106	C2	20000810	RU 1997-100857	19950623
EP 1088899	A2	20010404	EP 2001-200037	19950623
EP 1088899	A3	20010502		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV				
EP 1098006	A1	20010509	EP 2001-200045	19950623
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV				
EP 1098007	A1	20010509	EP 2001-200046	19950623
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				

EP 1091004 A2 20010411 EP 2001-200042 19950624

EP 1091004 A3 20010418

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE

US 6025132 A 20000215 US 1996-765332 19961223

AU 9935035 A1 19990902 AU 1999-35035 19990615

AU 723742 B2 20000907

US 6312903 B1 20011106 US 1999-448894 19991129

PRAI EP 1994-870106 A 19940624

EP 1995-870032 A 19950407

AU 1995-29246 A3 19950623

EP 1995-924923 A3 19950623

WO 1995-EP2452 W 19950623

US 1996-765332 A3 19961223

AB A method is presented for detection and identification of at least one microorganism, or for the simultaneous detection of several microorganisms in a sample. The polynucleic acids present in the sample may need to be released, isolated, or concd., and if necessary, the 16S-23S rRNA spacer region, or a part of it, is amplified with at least one suitable primer pair. The polynucleic acids are hybridized with at least one and preferably more than one of the 112 spacer probes or their equiv., under the appropriate hybridization and wash conditions, and/or with a taxon-specific probe derived from any of 103 spacer sequences under the same hybridization and wash conditions. The hybrids formed with each of the probes used under appropriate hybridization and wash conditions are detected, the microorganism(s) present in the sample identified from the differential hybridization signals obtained.

IT Acinetobacter

Acinetobacter baumannii

Bordetella pertussis

Brucella

Campylobacter

Cerebrospinal fluid

Chlamydia

Chlamydia psittaci

Chlamydia trachomatis

Digestive tract

Food analysis

Genitourinary tract

Haemophilus ducreyi

Haemophilus influenzae

Listeria

Listeria monocytogenes

Microorganism

Moraxella catarrhalis

Mycobacterium

Mycobacterium avium

Mycobacterium celatum

Mycobacterium chelonae

Mycobacterium fortuitum

Mycobacterium genavense

Mycobacterium gordonae

Mycobacterium haemophilum

Mycobacterium intracellulare

Mycobacterium kansasii

Mycobacterium malmoense

Mycobacterium marinum  
Mycobacterium paratuberculosis  
Mycobacterium scrofulaceum  
Mycobacterium simiae  
Mycobacterium tuberculosis  
Mycobacterium ulcerans  
Mycobacterium xenopi  
\*\*\*Mycoplasma\*\*\*  
\*\*\*Mycoplasma\*\*\* genitalium  
\*\*\*Mycoplasma\*\*\* pneumoniae

Neisseria gonorrhoeae  
Neisseria meningitidis  
Pseudomonas  
Pseudomonas aeruginosa  
Respiratory tract  
Salmonella  
Staphylococcus  
Staphylococcus aureus  
Staphylococcus epidermidis  
Streptococcus  
Streptococcus agalactiae  
Streptococcus pneumoniae  
Yersinia enterocolitica  
(simultaneous detection, identification and differentiation of  
eubacterial taxa using a hybridization assay based on the 16 S-23 S  
rRNA spacer region)

IT 174723-96-7

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(probe \*\*\*MHP\*\*\* -ICG-1; simultaneous detection, identification and  
differentiation of eubacterial taxa using a hybridization assay based  
on the 16 S-23 S rRNA spacer region)

IT 174696-41-4

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(probe \*\*\*Mycoplasma\*\*\* -ICG; simultaneous detection, identification  
and differentiation of eubacterial taxa using a hybridization assay  
based on the 16 S-23 S rRNA spacer region)

L10 ANSWER 19 OF 26 USPATFULL

AN 96:94338 USPATFULL

TI Inactivated \*\*\*Mycoplasma\*\*\* hypopneumoniae bacterin and method of  
use thereof

IN Petersen, Gary R., Lakeville, MN, United States  
Dayalu, K. I., Lincoln, NE, United States<sup>4</sup>)

PA Solvay Animal Health, Inc., Mendota Heights, MN, United States (U.S.  
corporation)

PI US 5565205 19961015

AI US 1990-568427 19900816 (7)

DT Utility

FS Granted

EXNAM Primary Examiner: Kim, Kay K. A.

LREP White, John P.

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN 2 Drawing Figure(s); 2 Drawing Page(s)

LN.CNT 1095



CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a bacterin comprising a virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, in an amount effective to immunize a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae and a suitable physiologically acceptable carrier. The invention also provides a method of producing this bacterin. The invention further provides a method of inactivating a virulent strain of \*\*\*Mycoplasma\*\*\* hyopneumoniae by contacting the \*\*\*Mycoplasma\*\*\* hyopneumoniae with binary ethyleneimine. Finally, the invention also provides a method of immunizing swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae comprising administering to the swine a dose of the bacterin so as to immunize the swine.

TI Inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae bacterin and method of use thereof

AB This invention provides a bacterin comprising a virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, in an amount effective to immunize a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae and a suitable physiologically acceptable carrier. The invention also provides a method of producing this bacterin. The invention further provides a method of inactivating a virulent strain of \*\*\*Mycoplasma\*\*\* hyopneumoniae by contacting the \*\*\*Mycoplasma\*\*\* hyopneumoniae with binary ethyleneimine. Finally, the invention also provides a method of immunizing swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae comprising administering to the swine a dose of the bacterin so as to immunize the swine.

SUMM \*\*\*Mycoplasma\*\*\* hyopneumoniae is a ubiquitous swine respiratory pathogen. It infects the respiratory tract of swine, colonizing the trachea, bronchi, and bronchioles. The \*\*\*mycoplasma\*\*\* produces a ciliostatic factor which causes the cilia lining the respiratory passages to stop beating. Eventually, the cilia degenerate, leaving the pig prone to infection by more serious secondary pathogens. The disease caused by \*\*\*Mycoplasma\*\*\* hyopneumoniae, enzootic pneumonia, is a chronic non-fatal disease which affects swine of all ages (R. F. Ross, \*\*\*Mycoplasmal\*\*\* diseases, pp. 436-444, in A. D. Laman, et al., (eds.) Diseases of Swine, Iowa State University Press, 1986). Infected pigs. . . one of the most important causes of disease-associated loss in swine (Whittlestone, pp. 133-176, in Tully and Whitcomb (eds.), The \*\*\*Mycoplasma\*\*\* Vol 2: Human and Animal \*\*\*Mycoplasmas\*\*\*, New York, Academic Press, (1979)). The disease generally results in inefficient weight gainers, and in stunted and sickly animals. Also, .

SUMM There have been numerous attempts to provide a vaccine for protecting swine against \*\*\*mycoplasmal\*\*\* pneumonia. However, such vaccines have not been successful, and the disease remains widespread.

SUMM Several investigators have disclosed vaccines comprising recombinantly produced surface antigens of \*\*\*Mycoplasma\*\*\* hyopneumoniae, Schaller et al., U.S. Pat. No. 4,894,332, issued Jan. 16, 1990; European Patent Publication No. 283,840, published Sep. 28, . . .

SUMM PCT Publication No. WO 86/00019, published Jan. 3, 1986, discloses a \*\*\*Mycoplasma\*\*\* hyopneumoniae vaccine comprising exclusively \*\*\*Mycoplasma\*\*\* hyopneumoniae plasma membranes, free of other cell components.

SUMM Etheridge et al., Res. Vet. Sci. 33: 188 (1982), found incomplete

protection against lung colonization by \*\*\*Mycoplasma\*\*\* hyopneumoniae when a live vaccine was given intravenously, subcutaneously, or intraperitoneally.

SUMM Kristensen et al., Am. J. Vet. Res. 42:784 (1981), found no protection of swine against \*\*\*mycoplasmal\*\*\* pneumonia after injection with heat-inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae.

SUMM Ross et al., Am. J. Vet. Res 45:1899 (1984), found that use of \*\*\*Mycoplasma\*\*\* hyopneumoniae extracts prepared by a freeze-thaw procedure to immunize swine, provided only variable protection, and in some instances, enhanced lesion. . . swine. These investigators also studied a whole-cell vaccine prepared by formalin inactivation. Formalin inactivation significantly hindered the protective immunogenicity of \*\*\*Mycoplasma\*\*\* hyopneumoniae, and this vaccine was not effective.

SUMM Yoshioka et al., U.S. Pat. No. 3,917,819, issued Nov. 4, 1975, discloses several killed \*\*\*Mycoplasma\*\*\* vaccines comprising \*\*\*Mycoplasma\*\*\* inactivated with formalin, including an inactivated vaccine for \*\*\*Mycoplasma\*\*\* hyopneumoniae. However, this vaccine was prepared by formalin inactivation.

SUMM Thus, prior studies with inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae vaccines have been unsuccessful, presumably because the conditions employed, i.e., heat treatment, formalin treatment, or freeze-thawing, destroyed the protective. . .

SUMM Accordingly, an effective whole cell vaccine for protecting swine against \*\*\*mycoplasmal\*\*\* pneumonia comprising inactivated virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae has not been developed heretofore.

SUMM This invention provides a whole-cell \*\*\*Mycoplasma\*\*\* hyopneumoniae vaccine or bacterin comprising \*\*\*Mycoplasma\*\*\* hyopneumoniae inactivated by treatment with binary ethyleneimine. In contrast with other techniques of inactivation, this method unexpectedly does not adversely. . .

SUMM This invention provides a bacterin comprising a virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, in an amount effective to immunize a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae, and a suitable physiologically acceptable carrier.

SUMM This invention also provides a method of producing a bacterin which comprises growing a virulent isolate of \*\*\*Mycoplasma\*\*\* hyopneumoniae in culture on a suitable medium; treating the virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae so grown with binary ethyleneimine so as to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae; concentrating the resulting, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae; recovering the resulting concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae; and admixing the concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae so recovered with a suitable physiologically acceptable carrier so as to formulate the bacterin.

SUMM This invention also provides a method of inactivating a virulent strain of \*\*\*Mycoplasma\*\*\* hyopneumoniae which comprises contacting a culture of \*\*\*Mycoplasma\*\*\* hyopneumoniae with binary ethyleneimine at a concentration of about 4 mM; incubating the culture under conditions effective to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae; and neutralizing the binary ethyleneimine in the culture by the addition of sodium thiosulfate at an effective neutralizing concentration.

SUMM This invention also provides a method of immunizing a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae comprising administering

to the swine a dose of a bacterin in accordance with the invention so as to immunize the swine against \*\*\*Mycoplasma\*\*\* hyopneumoniae infection.

DRWD FIG. 1. ELISA results comparing the relative antibody titers induced in pigs by a bacterin made by inactivation of \*\*\*Mycoplasma\*\*\* hyopneumoniae with binary ethyleneimine as compared with a bacterin made by inactivation with formalin.

DETD This invention provides a bacterin comprising a virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, in an amount effective to immunize a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae and a suitable physiologically acceptable carrier. In a presently preferred embodiment of this invention, the \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate of the bacterin comprises the isolate designated 1002 (ATCC Accession No. 55088).

DETD Although the present invention is described hereinafter with reference to the \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate designated 1002 (ATCC Accession No. 55088), it is contemplated that any virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, may be formulated into an effective bacterin. Numerous virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolates are known in the art and are available from various sources including the American Type Culture Collection. Finally, . . .

DETD The bacterin of the invention comprises the virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate, inactivated with binary ethyleneimine, in an amount effective to immunize a swine. To "effectively immunize" a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae means that the bacterin prevents or reduces the severity of \*\*\*mycoplasmal\*\*\* pneumonia. In any event, those skilled in the art are able to readily determine for any specific isolate and carrier combination, with and without an adjuvant being present, the amount of \*\*\*Mycoplasma\*\*\* hyopneumoniae, inactivated with binary ethyleneimine, effective to immunize a swine. In the presently preferred embodiment of the invention, the effective amount is at least about 10.sup.9 \*\*\*Mycoplasma\*\*\* hyopneumoniae DNA cell equivalents per milliliter of bacterin. (See Methods and Materials, hereinafter, for a discussion of "DNA cell equivalents".)

DETD This invention also provides a method of producing a bacterin which comprises growing a virulent isolate of \*\*\*Mycoplasma\*\*\* hyopneumoniae in culture on a suitable medium; treating the virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae so grown with binary ethyleneimine so as to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae; concentrating the resulting, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae; recovering the resulting concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae; and admixing the concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae so recovered with a suitable physiologically acceptable carrier so as to formulate the bacterin. In a preferred embodiment of the invention, the \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate designated 1002 (ATCC Accession No. 55088) is used in formulating the bacterin.

DETD More specifically, a virulent isolate of \*\*\*Mycoplasma\*\*\* hyopneumoniae is grown in culture in a suitable medium containing \*\*\*Mycoplasma\*\*\* broth base, yeast extract, glucose, L-cysteine, ampicillin, thallium acetate, swine serum and deionized water. One such medium which is presently. . .

DETD . . . about 48 hours to about 144 hours, measured from the time of incubation to the time of harvest. The virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate so grown is then treated with binary ethyleneimine so as to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae. Thus, the culture of the isolate may be contacted with binary ethyleneimine at a concentration of about 1 to about 10 mM, e.g., about 4 mM. The culture is then incubated under conditions effective to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae, e.g., for at least about 12 hours at about 37.degree. C. The binary ethyleneimine in the culture is then. .

DETD In one embodiment of the invention, the binary ethyleneimine which is used to contact and inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae is formed in situ by adding L-bromoethylamine hydrobromide to the culture in an amount, which upon conversion to binary. . .

DETD The resulting, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae is then concentrated. Various methods are known in the art for concentrating such organisms. For example, the organisms may. . .

DETD The concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae which result are then recovered, again using methods well known in the art. Finally, the resulting concentrated, inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae so recovered is admixed with a suitable physiologically acceptable carrier so as to formulate the bacterin. Optimally, the admixture. . .

DETD The bacterin may also be produced by any of several modifications of the preceding method. For example, the \*\*\*Mycoplasma\*\*\* hyopneumoniae may be concentrated before inactivation, rather than after. Also, preparation of the bacterin may be carried out by repeating. . .

DETD The concentrated, binary ethyleneimine-inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae is thus formulated as a bacterin by adding a suitable carrier. A diluent, dye, or preservatives may also be. . . (w/v). Diluents such as saline, or other carriers well known in the art, may be used to dilute the inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae in formulating the bacterin. Preservatives may also be added. In one embodiment of the invention, thimerosal is added as. . .

DETD This invention also provides a method of inactivating a virulent strain of \*\*\*Mycoplasma\*\*\* hyopneumoniae which comprises contacting a culture of \*\*\*Mycoplasma\*\*\* hyopneumoniae with binary ethyleneimine at a concentration of about 4 mM; incubating the culture under conditions effective to inactivate the \*\*\*Mycoplasma\*\*\* hyopneumoniae, such as, for an effective time, e.g. about 12 hours, at an effective temperature, e.g. about 37.degree. C.; and. . .

DETD This invention also provides a method of immunizing a swine against infection by \*\*\*Mycoplasma\*\*\* hyopneumoniae comprising administering to the swine at least one dose of the bacterin so as to immunize the swine against \*\*\*Mycoplasma\*\*\* hyopneumoniae infection. The bacterin may, in principle, be administered through various routes. However, in the presently preferred embodiment of the. . . it is presently preferred that the bacterin dose comprise about 2 ml of the bacterin, each ml containing about 10.sup.9 \*\*\*Mycoplasma\*\*\* hyopneumoniae DNA cell equivalents. The bacterin is desirably administered twice to the swine; once at about one week, and once. . .

DETD A bacterin in accordance with the present invention, intended for administration by injection, comprises inactivated, virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae suspended in an inert physiologically acceptable liquid diluent. Suitable diluents include water, saline, or

any of many other diluents well known in the art. Advantageously, the \*\*\*Mycoplasma\*\*\* hyopneumoniae are suspended in a sterile diluent under aseptic conditions.

DETD . . . prior to use or as a composition ready for use. In the latter case, the concentration of the inactivated, virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae is at least about 10.sup.9 DNA cell equivalents per milliliter.

DETD The binary ethyleneimine-inactivated \*\*\*Mycoplasma\*\*\* hyopneumoniae of the present invention may also be used as a component of a vaccine containing one or more other active ingredients, i.e., antigenic substances capable of inducing a protective immune response against \*\*\*Mycoplasma\*\*\* hyopneumoniae or against other disease-causing agents.

DETD The microorganism used in the preparation of the bacterin is the virulent \*\*\*Mycoplasma\*\*\* hyopneumoniae isolate designated 1002 and deposited under ATCC Accession No. 55088. This strain, originally isolated from an infected lung by culturing a 10.sup.-2 dilution of lung homogenate in \*\*\*Mycoplasma\*\*\* broth, was passed in broth seven times to establish the Master Seed ("X").

DETD The identity of the Master Seed ("X") was established by plating on \*\*\*Mycoplasma\*\*\* Broth Agar and examining the growth for typical \*\*\*Mycoplasma\*\*\* hyopneumoniae morphology. \*\*\*Mycoplasma\*\*\* hyopneumoniae, Strain J, obtained from NVSL, was used as a reference for comparison. The identity of the Master Seed ("X") was further established by SDS-PAGE and immunoblot comparison with References \*\*\*Mycoplasma\*\*\* hyopneumoniae, Strain J, and other related \*\*\*Mycoplasma\*\*\* species.

DETD 1. \*\*\*Mycoplasma\*\*\* Broth Base (Commercial) . . . 16.800 grams

DETD . . . been calibrated using DNA standards. The amount of fluorescence is proportional to the amount of DNA present. The number of \*\*\*Mycoplasma\*\*\* hyopneumoniae DNA cell equivalents (MHDCE) in the culture is calculated using the following formula: ##EQU1##

DETD The \*\*\*mycoplasmas\*\*\* are inactivated by exposure to binary ethyleneimine. The binary ethyleneimine (BEI) (primary inactivating agent) generated in situ during the incubation. . .

DETD The concentration of \*\*\*Mycoplasma\*\*\* cells in the final product is no less than 2.times.10.sup.9 \*\*\*Mycoplasma\*\*\* hyopneumoniae DNA cell equivalent per 2.0-ml dose, as determined by the DNA-fluorometric assay.

DETD Satisfactory \*\*\*Mycoplasma\*\*\* concentrates are aseptically combined with the adjuvant, preservative, dye, and diluent into a sterile container equipped with an agitator and. . .

DETD The virulence of four different strains of \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ): \*\*\*Mhp\*\*\* 1002-strain, \*\*\*Mhp\*\*\* 1005-NB 12 strain, \*\*\*Mhp\*\*\* 1003-J Strain, \*\*\*Mhp\*\*\* 1004-11 strain, and \*\*\*Mhp\*\*\* 1006-415 strain were evaluated.

DETD A low-passage stock culture of each of the four strains of \*\*\*Mycoplasma\*\*\* hyopneumoniae was thawed and a 10% inoculum of each strain was added to Pleuropneumonia-like organism (PPLO) complete medium (see page. . .

DETD There appeared to be no correlation between the dosage (CCU/pig) and the extent of lung lesions. M. hyopneumoniae 1002 and \*\*\*Mhp\*\*\* 1005-NB12 appeared to be the most virulent. However, M. hyopneumoniae 1002 grew faster and to a higher CCU level in. . .

DETD TABLE 1

Lung Lesion Involvement Resulting From Intranasal  
Inoculation of Four Different  
Strains of \*\*\*Mycoplasma\*\*\* hyopneumoniae  
M. hyopneumoniae Total No. Infected/  
strain CCU/Pig Total

1002 9 .times. 10.sup.7  
4/8 (50%)

1005 - NB12 9 .times. 10.sup.6  
4/8 (50%)

1003 -. . .

DETD \*\*\*MHP\*\*\* complete medium

DETD Binary ethyleneimine (BEI)-inactivated and formalin-inactivated  
\*\*\*Mycoplasma\*\*\* hyopneumoniae bacterins were evaluated for their  
ability to protect pigs against a virulent challenge with M.  
hyopneumoniae.

DETD \*\*\*Mycoplasma\*\*\* hyopneumoniae strain 1002, originally designated  
P-5723-3 (Dr. Charles Armstrong, Purdue University), was successively  
passed in a medium as described above. . .

DETD At the end of the incubation of the X+7 passage culture the extent of  
growth and concentration of \*\*\*mycoplasma\*\*\* were evaluated visually  
by the color of the culture medium and phase contrast microscopy,  
respectively. A sample was taken to. . .

DETD . . . at 37.degree. C. At the end of the incubation of the X+2  
passage, the extent of growth and concentration of \*\*\*Mycoplasma\*\*\*  
were evaluated in the same manner as the \*\*\*mycoplasma\*\*\*  
concentration for the vaccine. On the same day that animals received the  
primary vaccination, several one-week-old pigs were inoculated with. .

DETD . . . repeated on the following two days using the same culture. A  
sample of the 72-hour culture was quantitated for viable  
\*\*\*Mycoplasma\*\*\* present as determined by CCU. The titer was between  
5.times.10.sup.8 and 5.times.10.sup.9 CCU/ml. At the time of challenge,  
three to. . .

DETD These results suggest that circulating antibodies do not play a major  
role in immunoprotection against infection by \*\*\*Mycoplasma\*\*\*  
hyopneumoniae. Local secretory antibody and/or cell-mediated immunity  
are probably far more important. The BEI-inactivated bacterin appears to  
properly invoke these. . .

DETD The \*\*\*Mycoplasma\*\*\* hyopneumoniae bacterin was evaluated in a  
duration of immunity study. The bacterin was administered to pigs at 1  
and 3. . .

DETD The bacterin used in this study contained 9.0 log.sub.10  
\*\*\*Mycoplasma\*\*\* hyopneumoniae DNA cell equivalents (MHDCE) per  
milliliter.

DETD In this example, \*\*\*Mycoplasma\*\*\* hyopneumoniae bacterins containing  
7.0, 8.0, 9.0, 9.25 and 10.0 log.sub.10 M. hyopneumoniae DNA cell  
equivalents (MHDCE) per milliliter were evaluated. . .

DETD . . . the ELISA. Therefore, it is difficult to eliminate the  
possibility that some of these maternally derived antibodies are  
directed against \*\*\*Mycoplasma\*\*\* flocculare. M. hyopneumoniae and  
M. flocculare share some antigens. Nevertheless, these maternal  
antibodies appear to rapidly disappear and by 3. . .

DETD A fluorometric assay for measuring \*\*\*Mycoplasma\*\*\* hyopneumoniae

using the DNA binding dye Hoechst 33258 is described. The assay involves centrifugation of an *M. hyopneumoniae* culture to. . .

DETD One problem associated with \*\*\*Mycoplasma\*\*\* *hyopneumoniae* research is the difficulty of monitoring the growth of the organism. Because plating efficiency varies greatly at different stages. . . a culture into tubes of fresh media. The dilutions are then incubated for 14 days at 37.degree. C. Tubes containing \*\*\*Mycoplasma\*\*\* *hyopneumoniae* exhibit an acidic pH shift. The titer of the original culture is expressed in terms of the highest dilution. . .

DETD . . . of 0.16-16 ug DNA per milliliter of culture. The genome of *M. hyopneumoniae* is approximately 5.times.10.sup.8 daltons (S. Razin, The \*\*\*Mycoplasmas\*\*\*. Microbiol. Rev. 42:414-470 (1978)). This corresponds to 8.times.10.sup.-10 ug DNA per cell. The lower limit of the fluorometric assay is. . .

DETD . . . readings. The MHDCE are 4-5 times higher than the CCU values. It is generally felt that the CCU assay underestimates \*\*\*Mycoplasma\*\*\* concentrations. The DNA assay may therefore more accurately reflect the true number of *M. hyopneumoniae* cells. The CCU assay is. . .

DETD The CCU assay for quantitating *M. hyopneumoniae* suffers from several drawbacks. First, it probably underestimates the number of \*\*\*mycoplasma\*\*\* cells. Second, it is prone to experimental error. Large numbers of replicate tubes, expensive medium, and labor are required to. . .

DETD The DNA assay overcomes all of these problems. \*\*\*Mycoplasma\*\*\* cell estimates are higher than CCU estimates by 4-5 times and more accurately reflect the true cell count. When the. . . is required if several samples are to be analyzed). This makes the DNA assay appropriate for real time monitoring of \*\*\*mycoplasma\*\*\* cultures. It should be remembered that the DNA assay measures all cells regardless of whether they are living or dead. . .

DETD The assay is therefore most informative during the log phase of the \*\*\*mycoplasma\*\*\* growth curve.

CLM What is claimed is:

1. A bacterin comprising virulent \*\*\*Mycoplasma\*\*\* *hyopneumoniae* isolate 1002 (Strain P-5723-3/ ATCC Accession No. 55088), inactivated with binary ethyleneimine, in an amount not less than 10.sup.9 \*\*\*Mycoplasma\*\*\* *hyopneumoniae* DNA cell equivalents per milliliter of bacterin, a suitable physiologically acceptable carrier and an adjuvant.
4. A method of immunizing a swine against infection by \*\*\*Mycoplasma\*\*\* *hyopneumoniae* which comprises administering to the swine the bacterin of claim 1 so as to immunize the swine against \*\*\*Mycoplasma\*\*\* *hyopneumoniae* infection.

L10 ANSWER 20 OF 26 CAPLUS COPYRIGHT 2002 ACS

AN 1996:277294 CAPLUS

DN 124:334063

TI Oligonucleotide probes complementary to 16S rRNA

AU Johansson, Karl-Erik

CS Laboratory Bacteriology, Research and Development, National Veterinary Institute, Uppsala, S-750 07, Swed.

SO Molecular and Diagnostic Procedures in Mycoplasma (1996), Volume 2, 29-46. Editor(s): Tully, Joseph G.; Razin, Shmuel. Publisher: Academic,

San Diego, Calif.  
CODEN: 62QDA9

DT Conference

LA English

AB Lethal yellowing (LY) is a fast spreading, highly destructive disease of coconut palms and 33 addnl. palm species. This disease presently occurs in several countries of the western Caribbean region, where it has virtually eliminated the susceptible Atlantic Tall ecotype from many localities. A \*\*\*mycoplasma\*\*\* -like organism (MLO) is believed to be the causal agent of LY. The LY MLO, like all other plant pathogenic MLOs, remains unculturable and thus precludes culture techniques for diagnosis. Diagnosis of LY has relied primarily upon symptom identification. Unequivocal disease diagnosis requires further visual conformation of the presence of MLOs in affected palm tissues. This has traditionally been accomplished by either electron microscopy of DAPI staining in conjunction with epifluorescence microscopy. Although quite sensitive, both techniques are non-specific since they cannot distinguish one MLO from another. DNA-based techniques have recently been developed which enable both sensitive and specific detection of the LY MLO. The authors present oligonucleotide probes complementary to 16S rRNA for MLO detection and discrimination.

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IT Deoxyribonucleic acid sequences

(of oligonucleotide probes for detection and identification of

\*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT \*\*\*Mycoplasma\*\*\*

(-like organisms, detection of; oligonucleotide probes complementary to 16S rRNA)

IT 135116-62-0

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe \*\*\*MHP1\*\*\* for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 135116-63-1

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe \*\*\*MHP2\*\*\* for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide



probes complementary to 16S rRNA)

IT 176592-94-2

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe \*\*\*MHP3\*\*\* for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 144245-08-9

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe MP20 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 176592-86-2

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe MP30 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 176592-89-5

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mag30 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 176592-87-3

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mbg28 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 176592-88-4

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mbo29 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 147759-24-8

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mcc1 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 147759-25-9

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mcc2 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

IT 147759-26-0

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence of oligonucleotide probe Mcc3 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

- IT 176592-95-3  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe MgaV7/31 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)
- IT 176592-91-9  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe \*\*\*Mhp3\*\*\* /32 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)
- IT 176592-92-0  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe \*\*\*Mhp6\*\*\* /30 detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)
- IT 176592-90-8  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe \*\*\*Mhp8\*\*\* /24 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)
- IT 176592-93-1  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe Mhy2/27 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)
- IT 176592-96-4  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (nucleotide sequence of oligonucleotide probe MsyV8/31 for detection and identification of \*\*\*Mycoplasmas\*\*\* ; oligonucleotide probes complementary to 16S rRNA)

L10 ANSWER 21 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

5

AN 1994:272946 BIOSIS

DN PREV199497285946

TI An improved enzyme linked immunosorbent assay (ELISA) for the detection of porcine serum antibodies against \*\*\*Mycoplasma\*\*\* hyopneumoniae.

AU Djordjevic, S. P. (1); Eamens, G. J.; Romalis, L. F.; Saunders, M. M.

CS (1) Elizabeth Macarthur Agric. Inst., NSW Agric., PMB 8, Camden, NSW 2750 Australia

SO Veterinary Microbiology, (1994) Vol. 39, No. 3-4, pp. 261-273.  
 ISSN: 0378-1135.

DT Article

LA English

AB An ELISA for the detection of serum antibodies to \*\*\*Mycoplasma\*\*\* hyopneumoniae in pigs and based on a 43 kDa purified protein derived from the cytoplasmic membrane of M. hyopneumoniae strain J is described. This ELISA ( \*\*\*MHPP\*\*\* ELISA) was compared with another recently described (Auspharm ELISA, Sheldrake and Romalis 1992) that is based on

column-purified sonicated proteins of strain J. Using sample to negative ELISA ratios of 3 and 4 as cutoffs for inconclusive and positive reactors respectively (compared to 2 and 3 for the Auspharm ELISA), the two tests had high specificity ( \*\*\*MHPP\*\*\* 99.6% Auspharm 100%) in 280 SPF pigs. In 176 pigs from commercial herds with endemic *M. hyopneumoniae*, the \*\*\*MHPP\*\*\* ELISA showed a higher sensitivity than the Auspharm ELISA in both high lung score (LS gtoreq 5) (85.5% vs. 69.9%) and low lung score (0 lt LS lt 5) (57.9% vs. 49%) pigs when the positive cutoff for each test was selected. The sensitivity when the inconclusive cutoff was selected was similar in both tests (85.5%; 85.7%) when low and high lung score pigs were pooled. Although the \*\*\*MHPP\*\*\* also gave more positive reactors in 36 pigs from *M. hyopneumoniae*-infected herds with no lung pathology at slaughter than the Auspharm ELISA (11 vs. 4), the total number of inconclusive and positive reactors in these pigs was similar for both tests (11 vs. 14). The \*\*\*MHPP\*\*\* ELISA gave significantly higher ELISA ratios in infected pigs (up to 17.9) than the Auspharm ELISA (up to 9), and earlier seroconversion in naturally-infected (6-8 weeks vs. 9-10 weeks) and experimentally-infected pigs (2-4 weeks vs. 4-6 weeks post infection).

TI An improved enzyme linked immunosorbent assay (ELISA) for the detection of porcine serum antibodies against \*\*\*Mycoplasma\*\*\* hyopneumoniae.

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BC \*\*\*Mycoplasmataceae\*\*\* 07512  
Suidae \*85740

ORGN Super Taxa

\*\*\*Mycoplasmataceae\*\*\* : Eubacteria, Bacteria; Suidae: Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name

\*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mycoplasmataceae\*\*\* ); Suidae (Suidae)

ORGN Organism Superterms

animals; artiodactyls; bacteria; chordates; eubacteria; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; vertebrates

TI Development of polymerase chain reaction primers to detect

\*\*\*Mycoplasma\*\*\* hyopneumoniae

AU Artiushin, S.; Stipkovits, L.; Minion, F. C.

CS Vet. Med. Res. Inst., Iowa State Univ., Ames, IA, USA

SO Molecular and Cellular Probes (1993), 7(5), 381-5

CODEN: MCPRE6; ISSN: 0890-8508

DT Journal

LA English

AB The purpose of this study was to develop a species-specific polymerase chain reaction (PCR)-based diagnostic reagent for the identification of \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ). Based upon DNA sequence anal. of a cloned fragment of \*\*\*Mhp\*\*\* DNA, PCR primers were constructed and tested against different strains of \*\*\*Mhp\*\*\* , \*\*\*Mycoplasma\*\*\* flocculare, other \*\*\*mycoplasma\*\*\* species, and non-Mollicute micro-organisms which commonly inhabit the respiratory tracts of swine. A total of 40 field isolates from \*\*\*Mhp\*\*\* and four field isolates of M. flocculare have been examd. Pos. signals were obtained in PCR with \*\*\*Mhp\*\*\* ref. strains and all 40 \*\*\*Mhp\*\*\* field isolates, but not with other Mollicutes or micro-organisms.

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ST polymerase chain reaction detection \*\*\*Mycoplasma\*\*\*

IT Polymerase chain reaction

(for detection of \*\*\*Mycoplasma\*\*\* hyopneumoniae)

IT \*\*\*Mycoplasma\*\*\* hyopneumoniae

(polymerase chain reaction for detection of)

IT 154339-48-7 154339-49-8

RL: USES (Uses)

(as DNA primer of polymerase chain reaction, for detection of  
\*\*\*Mycoplasma\*\*\* hyopneumoniae)

L10 ANSWER 23 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

7

AN 1992:348717 BIOSIS

DN BA94:40942

TI DETECTION OF \*\*\*MYCOPLASMA\*\*\* -HYOPNEUMONIAE BY USING RRNA  
OLIGODEOXYNUCLEOTIDE HYBRIDIZATION.

AU FUTO S; SETO Y; MITSUSE S; MORI Y

CS CENTRAL LABORATORY, NIPPON FLOUR MILLS CO. LTD., ATSUGI, KANAGAWA, JPN.

SO J CLIN MICROBIOL, (1992) 30 (6), 1509-1513.

CODEN: JCMIDW. ISSN: 0095-1137.

FS BA; OLD

LA English

AB A system that uses rRNA-oligodeoxynucleotide hybridization was developed

for the detection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. Synthetic oligonucleotide \*\*\*MHP1\*\*\* was hybridized specifically with M. hyopneumoniae. Furthermore, the detection of M. hyopneumoniae in clinical samples, such as bronchoalveolar lavage fluid and lung lesions from experimentally infected pigs, was evaluated by this assay. The evidence obtained from the assay indicated that the system can be used to efficiently diagnose \*\*\*mycoplasmal\*\*\* pneumonia of swine. Additionally, a nonradioisotopic system with chemiluminescence detection was tested. This system was 10-fold less sensitive than a test that used radioisotopes.

TI DETECTION OF \*\*\*MYCOPLASMA\*\*\* -HYOPNEUMONIAE BY USING RRNA OLIGODEOXYNUCLEOTIDE HYBRIDIZATION.

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BC \*\*\*Mycoplasmataceae\*\*\* 07512  
Suidae 85740

IT Miscellaneous Descriptors

BRONCHOALVEOLAR LAVAGE FLUID EXPERIMENTALLY INFECTED PIG LUNG LESION  
\*\*\*MYCOPLASMAL\*\*\* PNEUMONIA RIBOSOMAL RNA RADIOISOTOPIC VS.  
CHEMILUMINESCENT DETECTING SYSTEM SENSITIVITY MOLECULAR DIAGNOSTIC  
METHOD

L10 ANSWER 24 OF 26 CABA COPYRIGHT 2002 CABI

AN 95:182035 CABA

DN 952215118

TI Control of porcine enzootic pneumonia ( \*\*\*mycoplasma\*\*\* pneumonia of swine) induced by \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ) using an inactivated adjuvanted vaccine

AU Dayalu, K. I.; Keich, R. L.; Bhogal, B. S.; Landicho, E. F. [EDITOR]; Maala, C. P. [EDITOR]; Mateo, A. A. B. [EDITOR]; Vilacorte, E. Z. V. [EDITOR]

SO Proceedings 8th Congress of the Federation of Asian Veterinary Associations, 21-25 November 1992, The Manila Hotel Manila, Philippines, (1992) pp. 254-260.

Publisher: Philippine Veterinary Medical Association. Quezon City  
Meeting Info.: Proceedings 8th Congress of the Federation of Asian Veterinary Associations, 21-25 November 1992, The Manila Hotel Manila, Philippines.

ISBN: 971-91326-0-4

CY Philippines

DT Conference Article

LA English

TI Control of porcine enzootic pneumonia ( \*\*\*mycoplasma\*\*\* pneumonia of swine) induced by \*\*\*Mycoplasma\*\*\* hyopneumoniae ( \*\*\*Mhp\*\*\* ) using an inactivated adjuvanted vaccine.

BT Sus; Suidae; Suiformes; Artiodactyla; ungulates; mammals; vertebrates; Chordata; animals; Sus scrofa; \*\*\*Mycoplasma\*\*\* ;  
\*\*\*Mycoplasmataceae\*\*\* ; \*\*\*Mycoplasmatales\*\*\* ; Mollicutes;

Tenericutes; bacteria; prokaryotes  
ORGN pigs; \*\*\*Mycoplasma\*\*\* hyopneumoniae

L10 ANSWER 25 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE  
8

AN 1992:330631 BIOSIS

DN BA94:32472

TI SPECIFICITY OF OLIGONUCLEOTIDE PROBES COMPLEMENTARY TO EVOLUTIONARILY  
VARIABLE REGIONS OF 16S RRNA FROM \*\*\*MYCOPLASMA\*\*\* -HYOPNEUMONIAE AND  
\*\*\*MYCOPLASMA\*\*\* -HYORHINIS.

AU JOHANSSON K-E; MATTSSON J G; JACOBSSON K; FERNANDEZ C; BERGSTROM K; BOLSKE  
G; WALLGREN P; GOBEL U B

CS NATL. VET. INST., LAB. BACTERIOL., BOX 7073, S-750 07 UPPSALA, SWED.

SO RES VET SCI, (1992) 52 (2), 195-204.

CODEN: RVTSA9. ISSN: 0034-5288.

FS BA; OLD

LA English

AB \*\*\*Mycoplasma\*\*\* is the common name for the smallest free-living  
microorganisms, the Mollicutes. \*\*\*Mycoplasma\*\*\* hyopneumoniae is of  
great importance in veterinary medicine, causing enzootic pneumonia in  
pigs. M. hyorhinis can cause polyserositis and may cause pneumonia in  
piglets. Oligonucleotides complementary to variable regions of 16S rRNA  
from these \*\*\*mycoplasmas\*\*\* were designed and used as probes for  
detection and identification of these \*\*\*mycoplasmas\*\*\*. The probe  
complementary to 16S rRNA of M. hyorhinis gave a very weak  
cross-hybridisation with M. hyosynoviae in filter hybridisation  
experiments, but not with any of the other porcine \*\*\*mycoplasmas\*\*\*  
tested. Three oligonucleotide probes complementary to M. hyopneumoniae 16S  
rRNA were tested. One of the probes ( \*\*\*Mhp6\*\*\* /30) was found to be  
specific to M. hyopneumoniae, but the other two gave cross-hybridisation  
with M. flocculare. Using the \*\*\*Mhp6\*\*\* /30 probe in direct filter  
hybridisation experiments, it proved possible to detect M. hyopneumoniae  
in lung biopsies from experimentally infected pigs.

TI SPECIFICITY OF OLIGONUCLEOTIDE PROBES COMPLEMENTARY TO EVOLUTIONARILY  
VARIABLE REGIONS OF 16S RRNA FROM \*\*\*MYCOPLASMA\*\*\* -HYOPNEUMONIAE AND  
\*\*\*MYCOPLASMA\*\*\* -HYORHINIS.

AB \*\*\*Mycoplasma\*\*\* is the common name for the smallest free-living  
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great importance in veterinary medicine, causing enzootic pneumonia in  
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in lung biopsies from experimentally infected pigs.

BC \*\*\*Mycoplasmataceae\*\*\* 07512  
Suidae 85740

L10 ANSWER 26 OF 26 JAPIO COPYRIGHT 2002 JPO

AN 2001-149085 JAPIO

TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae,  
PROTEIN ENCODED BY THE GENE, AND USES THEREOF

IN KING KENDALL WAYNE; MADURA REBECCA ANNE; ROSEY EVERETT LEE

PA PFIZER PROD INC

PI JP 2001149085 A 20010605 Heisei

AI JP 2000-300778 (JP2000300778 Heisei) 20000929

PRAI US 1999-156602 19990929

SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 2001

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae

\*\*\*mhp3\*\*\* gene nucleic acid and a protein encoded by the nucleic acid.

SOLUTION: A new apoprotein antigen is provided which is encoded by

\*\*\*mhp3\*\*\* used in vaccine for preventing and treating a disease caused

by the infection of \*\*\*Mycoplasma\*\*\* hyopneumoniae. A method for

producing the antigen by the recombination is also provided.

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TI NUCLEIC ACID OF \*\*\*mhp3\*\*\* GENE OF \*\*\*Mycoplasma\*\*\* hyopneumoniae,  
PROTEIN ENCODED BY THE GENE, AND USES THEREOF

AB PROBLEM TO BE SOLVED: To obtain \*\*\*Mycoplasma\*\*\* hyopneumoniae

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SOLUTION: A new apoprotein antigen is provided which is encoded by

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